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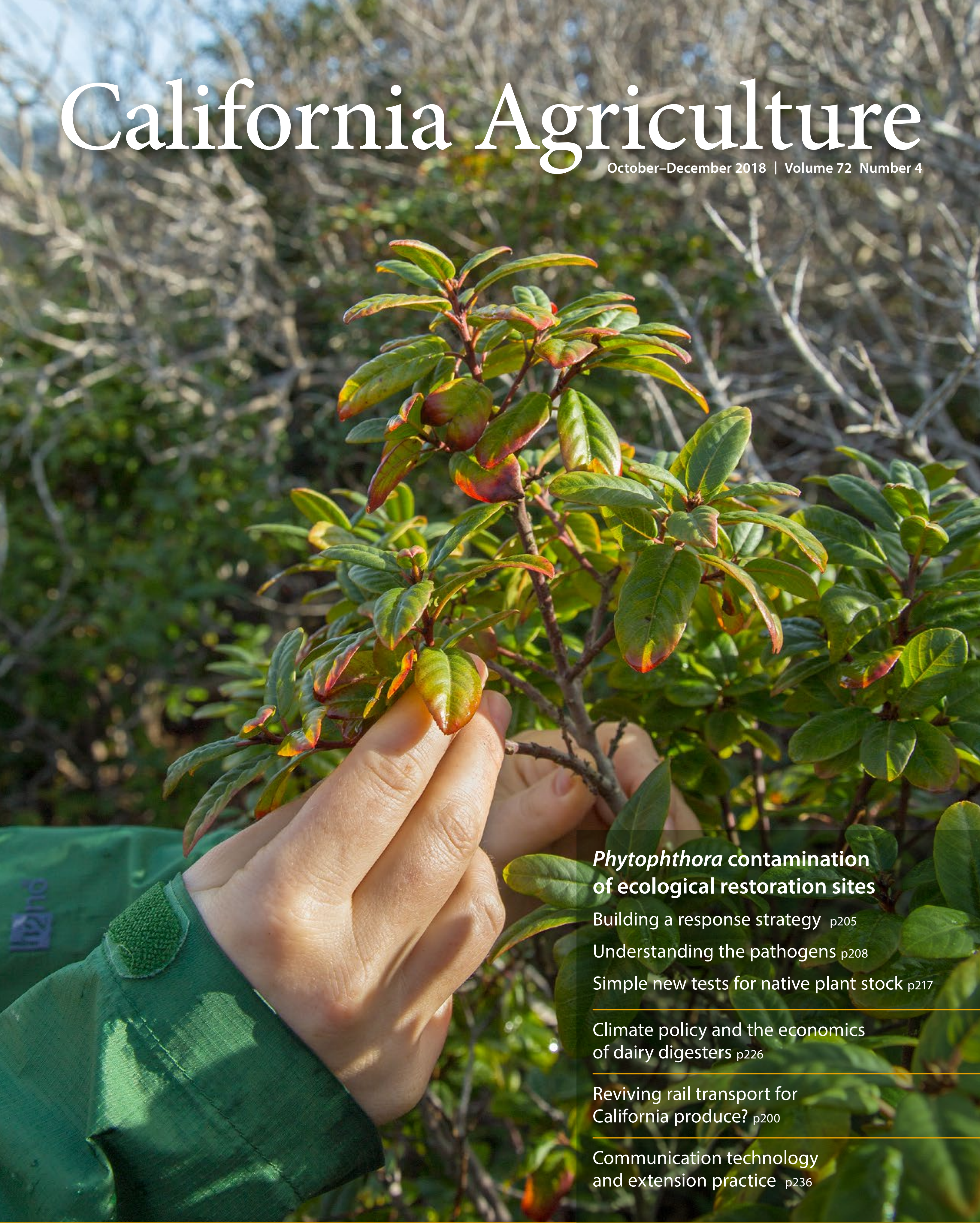

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California Agriculture

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ORDERS AND SUBSCRIPTIONS

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EDITORIAL

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UNIVERSITY OF CALIFORNIA

UNIVERSITY OF CALIFORNIA
Agriculture and Natural Resources

Contents

OCTOBER–DECEMBER 2018 • VOLUME 72, NUMBER 4

News and opinion

RESEARCH NEWS

OUTLOOK

Research and review articles

COVER: Leaves on a coffeeberry shrub (*Frangula californica*) display symptoms of infection by pathogens in the genus *Phytophthora* at an ecological restoration site on Mori Point, Pacifica, in San Mateo County. Three articles in this issue (pages 205, 208 and 217) address a serious concern for the restoration of natural areas in California: The inadvertent infestation of restoration sites with multiple *Phytophthora* species via infected planting stock. *Photo by Will Suckow.*

NEWS

Research highlights

Recent articles from the Agricultural Experiment Station campuses and UC ANR's county offices, institutes and research and extension centers

Extreme drought advantages locally rare species

Climatic volatility is altering ecosystems across the planet, but little research has attempted to quantify the effects of extreme climate events on the composition of ecological communities. A team of researchers led by Laura Prugh of the University of Washington, and including Justin Brashares of the Department of Environmental Science, Policy and Management at UC Berkeley, set out to redress this gap in research. Working in San Luis Obispo County's Carrizo Plain National Monument (a semi-arid grassland), they examined the responses of 423 species to California's 2012–2015 drought, California's driest period in the last 1,200 years.

The researchers categorized each species they studied as a "winner" or a "loser," depending on whether the species increased or decreased in abundance; a third category was established for species whose abundance was unaffected by the drought. Eighty-five species emerged as losers; 12 came out as winners; and 239 showed no significant response to the drought (87 species present for one year only were excluded). Winners included seven species of insect, one plant, one reptile, two birds and one rodent. Plants showed the most significant response to a single year of drought, whereas extended drought had its greatest impact on carnivorous animals.

The researchers report that locally rare species were more likely to "win" and abundant species more likely to "lose." This tendency, they say, was remarkably consistent across taxa and drought durations, suggesting that drought "indirectly promote[s] the long-term persistence of rare species by stressing dominant species throughout the food web." The researchers note that while extreme drought "can lead to substantial short-term declines in the abundance and diversity of species across taxonomic groups," such disturbances — by inducing occasional die-offs among dominant species and thus providing rare, fast-growing species with opportunities to thrive — "may play a vital role in the long-term maintenance of biodiversity."

Prugh LR, Deguines N, Grinath JB, et al. 2018. Ecological winners and losers of extreme climate change in California. Nat Clim Change 8(9):819–24. https://doi.org/10.1038/s41558-018-0255-1

Controlling fumigant emissions and nematodes with deep injections and biochar applications

Fumigation is a means of controlling harmful organisms in soil, including plant parasitic nematodes. During fumigation, liquids injected into the soil volatize into gas that spreads through the soil's air space. But these gases can also escape into the air, degrading air quality and potentially harming human health. Regulators have developed and continue to amend

Working in the Carrizo Plain National Monument, a semi-arid grassland in San Luis Obispo County, researchers examined the responses of 423 species to California's 2012–2015 drought, California's driest period in the last 1,200 years.

Bob Wick, BLM California

rules limiting fumigant emissions — and the plastic films sometimes used to control emissions carry significant costs. Growers' continued ability to practice fumigation may therefore depend on finding new ways to reduce emissions.

A group of researchers led by Suduan Gao of the U.S. Department of Agriculture and David Doll of UC Cooperative Extension (UCCE) in Merced County — and including (among others) Becky Westerdahl of the Department of Entomology and Nematology at UC Davis and Bradley Hanson of the Department of Plant Sciences at UC Davis — set out to determine if emissions could be reduced by injecting fumigants deeper in the soil than is customary. Building on previous research into soil-sealing techniques and materials, in this work they also sought to determine if applications of biochar, a carbon-rich substance derived from biomass, could further reduce fumigant emissions to the atmosphere. In addition, because pest control drives fumigation decisions, they sought to determine the effects of deep fumigation and biochar application on fumigant distribution in soil and on control of plant parasitic nematodes, which are often a cause of poor orchard establishment and performance.

Working at the site of a removed almond orchard in Stanislaus County, the researchers conducted experiments involving several variables: whether fumigant was injected at a standard depth (18 inches), or a deeper one (26 inches); whether it was applied at standard rates, or lower ones; and whether the soil was covered with plastic film or biochar, or not covered at all. Fumigant emissions were measured for more than a month after application and fumigant concentration in the soil pore space was measured to a depth of 125 centimeters for several months.

Results showed that deep injection enhanced fumigant delivery to depths below 60 centimeters while also resulting in significantly lower peak emissions compared to standard injection depth. Data also indicated that biochar amendments can significantly reduce fumigant emissions without reducing nematode control — though the researchers say that additional research is needed to validate results under a range of field conditions.

Gao S, Doll DA, Stanghellini MS, et al. 2018. Deep injection and the potential of biochar to reduce fumigant emissions and effects on nematode control. J Environ Manage 223:469–77. https://doi.org/10.1016/j.jenvman.2018.06.031

Measuring dispersal of grape pests' natural enemies from a buckwheat cover crop

Key pests affecting grapes in California include leafhoppers, mites and thrips. These pests' natural enemies include spiders, leafhopper parasitoids, predatory thrips and minute pirate bugs. When pests' natural enemies disperse from cover crops, they can provide varying levels of pest control in crops such as grapes. To determine the efficacy of cover crops for pest control, it is important to determine the distances over which natural enemies will move from cover crops and into high-value crops.

A team of researchers — led by Nicola Irvin, a biological control specialist and research scholar in the Department of Entomology at UC Riverside, and including Mark Hoddle, a biological control specialist and principal investigator in the same department — set out to determine the distances over which the natural enemies of grape pests will disperse when buckwheat is established as a cover crop in a vineyard. Working in a Southern California organic vineyard, the researchers sprayed flowering buckwheat cover crops with a "triple mark" solution containing yellow dye, casein protein and albumin protein. The researchers then placed transparent sticky traps at predetermined locations around the vineyard, capturing marked natural enemies for examination under a dissecting microscope.

Results showed that spiders, predatory thrips and minute pirate bugs dispersed 9 meters from marked buckwheat refuges. Twenty-two percent of marked leafhopper parasitoids were captured between 18 and 30 meters from marked buckwheat plots. Some arthropods were able to cross 36-meter buffer zones devoid of vegetation. According to the researchers, buckwheat refuges could therefore be planted in California vineyards every sixth or 10th row. They caution, however, that planting cover crops in Southern California's arid

Nicola A. Irvin, UC Riverside

Scientists with UC Riverside and USDA-ARS studied a Southern California organic vineyard to determine how far natural enemies of grape pests disperse when buckwheat is used as a cover crop. Results showed that spiders, predatory thrips and minute pirate bugs dispersed 9 meters from marked buckwheat refuges. The researchers suggest that buckwheat could be planted in California vineyards every sixth or 10th row, but caution that supplying water to the cover crop could lead to negative effects such as reduced brix levels and an increased risk of insect pest and disease prevalence.

grape production areas and supplying supplemental water to the cover crop could lead to negative effects such as increased risk of insect pest and disease prevalence, increased management costs, and reduced brix levels.

Irvin NA, Hagler JR, Hoddle MS. 2018. Measuring natural enemy dispersal from cover crops in a California vineyard. Biol Control 126:15–25. https://doi.org/10.1016/j.biocontrol.2018.07.008

Measuring chemical changes during aging of malbec wine

Phenolic compounds — a large group of secondary metabolites in plants — play a fundamental role in establishing a wine's sensorial characteristics, antioxidant capacity and ultimately its quality. Factors that affect a wine's concentration of phenolic compounds include grape variety, region of origin and duration of aging. Bottle aging, required for optimal quality in some red wines, allows diverse chemical reactions to occur, with phenolic compounds evolving and concentrations of elements changing. But no studies examining these chemical processes during the aging of malbec red wines had been conducted. A team of researchers led by Federico Agazzi of Argentina's Catena Institute of Wine took up this research topic, examining phenolic compounds and also the elemental changes that can affect a wine's stability during aging. The team included UC Davis Assistant Adjunct Professor Jenny Nelson (Department of Viticulture and Enology), Courtney Tanabe (doctoral candidate in the Agricultural and Environmental Chemistry Graduate Group at UC Davis), UC Davis Staff Research Associate Carolyn Doyle (Department of Viticulture and Enology) and UC Davis Professor Roger Boulton (Department of Viticulture and Enology and Department of Chemical Engineering).

To find out how the bottle aging process affects phenolic compounds and concentration of elements in red wines, a team of researchers compared wines produced from malbec grapes grown in Argentina and California. Their findings show that wines can be distinguished by region after five years of aging.

The team examined malbec wines produced from grapes grown in six districts in Argentina and seven districts in California. The team's research aims were to identify malbec's chemical fingerprint after five years of aging and to compare the chemical composition of aged malbec wine to that of malbec at the beginning of its aging process.

After five years the researchers could, when they assessed polyphenols and elemental data in combination, differentiate malbec wines by region. They observed that total polyphenol content was significantly affected by aging time. The potassium concentration of Argentine wines decreased over time while magnesium content increased. California wines showed a decrease in potassium and calcium and an increase in magnesium. Polyphenols and elemental concentrations, beyond their utility in distinguishing wines from different regions, can impact both the taste and appearance of aged wines — and thus the study's results might influence wineries' decisions regarding the aging of malbec wines.

Agazzi FM, Nelson J, Tanabe CK, et al. 2018. Aging of Malbec wines from Mendoza and California: Evolution of phenolic and elemental composition. Food Chem 269:103–10. https://doi.org/10.1016/j.foodchem.2018.06.142

Farmers hold nuanced views regarding on-farm food loss

Food loss and waste have gained increasing attention from academics, activists, entrepreneurs, policymakers and the public. Reducing total food loss, from farm to consumption, could help produce several desirable outcomes: reduced use of scarce environmental resources, improved food security, and increased income for farmers through secondary markets. Achieving such goals will require accurate assessments of the causes of food loss and creative thinking about potential remedies.

Past research on food waste has focused almost exclusively on food loss at the consumer and retail levels, with little research devoted to food loss on farms. Two UC Davis researchers — UCCE Specialist David Campbell (Department of Human Ecology) and Kate Munden-Dixon, a doctoral candidate in the Geography Graduate Group — embarked on an exploratory project focused on farm-based food loss. Their research goal was to better understand farmers' views on the nature and extent of on-farm food loss, on the causes of on-farm food loss and on food recovery strategies' potential to reduce loss.

In a small pilot study conducted in collaboration with the California Food Waste Roundtable, the researchers conducted interviews with representatives of 12 California fruit and vegetable operations, ranging from diversified fruit and vegetable farms of less than 10 acres to an export-oriented operation covering more than 30,000 acres. The researchers, analyzing the interviews, identified several themes: Growers find it difficult to provide precise estimates of how much food loss occurs on their farms, in part because loss varies significantly across different crops and growing seasons; losses are often caused by weather, unpredictable market conditions or cosmetic standards imposed by buyers; very little of the food lost on farms ends up in landfills because many unsold food items are either tilled into the ground, used as animal feed or donated to food banks; farmers can be hesitant to partner with food recovery groups due to liability concerns or to time constraints during busy harvest seasons; and growers believe that efforts to reduce food loss should focus on the processing sector, where they believe greater food recovery possibilities exist. The researchers recommend that policy efforts to reduce food loss take farmer perspectives into account and concentrate on crops with relatively high loss percentages that are also of high utility to food banks. A UC Davis research team led by Professor Edward Spang (Department of Food Science and Technology) is currently undertaking a more detailed study of this topic.

Campbell D, Munden-Dixon K. 2018. On-farm food loss: Farmer perspectives on food waste. J Extension 56(3). https://joe.org/joe/2018june/a5.php

Productivity growth on U.S. farms has slowed

Have U.S. farms exhibited slower rates of productivity growth in recent decades? This has been a contentious question among agricultural economists — and much depends on the answer. If U.S. farmers are to remain competitive in world markets, their farms must sustain a comparatively rapid rate of productivity growth. Meanwhile, around the world, food supplies and prices depend directly and indirectly on innovations in U.S. farming.

A research team led by Matthew Andersen of the University of Wyoming — and including UC Davis Professors Julian Alston and Aaron Smith (both of the Department of Agricultural and Resource Economics) — set out to determine through data analysis whether farm productivity growth in the United States has indeed slowed. Assembling a range of agricultural productivity measures, they performed a battery of statistical procedures and tests designed to investigate the nature of changes in the rate of productivity growth over many decades. Their results, they say, provide robust and compelling evidence that productivity in U.S. agriculture has recently undergone a structural slowdown. By one measure, over the final 10 to 20 years in the researchers' dataset, productivity grew at only half the rate that had been sustained for much of the 20th century. Of equal importance, the researchers say, is that the relatively rapid rates of productivity growth experienced during the 1960s, 1970s and 1980s can be construed as aberrations.

The researchers suggest that the slowdown in agricultural productivity might be related to an earlier slowdown in the growth of spending on agricultural research and development. They warn that failure to revive growth in U.S. agricultural productivity over the coming decades could carry serious consequences. Without renewed productivity growth, natural resource stocks will be depleted faster, less agricultural output will be produced and food will be more expensive than would have been the case with higher rates of productivity growth. The U.S. agricultural sector might suffer from diminished competitiveness with other countries. For example, farm productivity and spending on agricultural research and development have grown significantly in China and Brazil, relative to the United States, and these countries have grown in importance as agricultural producers. But if nations outside the United States experience their own slowdowns in agricultural productivity growth, a widening gap will separate growth in global demand for agricultural products from growth in global supply of those products.

Andersen MA, Alston JM, Pardey PG, Smith A. 2018. A century of U.S. farm productivity growth: A surge then a slowdown. Am J Agr Econ 100(4):1072–90. https://doi.org/10.1093/ajae/aay023

An analysis of U.S. agricultural production over the past 100 years indicates that the rate of productivity growth has slowed considerably. In the final 10 to 20 years of the researchers' dataset, farm productivity grew at only half the rate that had been sustained for much of the 20th century. The researchers suggest that the slowdown could be related to an earlier slowdown in the growth of spending on agricultural research and development.

OUTLOOK

Back on track? Reassessing rail transport for California's perishable produce

Moving perishable produce by rail, rather than by truck, could provide significant benefits for Californians.

Joshua Seeherman, Postdoctoral Researcher, California Partners for Advanced Transportation Technology (PATH), UC Berkeley

Karen Trapenberg Frick, Associate Professor, Department of City and Regional Planning, UC Berkeley

Juan Caicedo, Doctoral Student, Institute of Transportation Studies, UC Berkeley

Mark Hansen, Professor, Institute of Transportation Studies, UC Berkeley

Railroads have long been an important component of California's freight transportation network. For perishable produce in particular, the rail industry dominated until 1950 — but between 1960 and 1990, trucking took over. In 2016, California exported about $20 billion in produce (CDFA 2017). But only 3% of the state's exported perishable produce travels a majority of the distance to its destination by rail.

Transport of perishable produce has shifted from rail to trucking for complex reasons, but the change has not been altogether beneficial for Californians. Indeed, several negative externalities are associated with the truck-based transport of the overwhelming majority of the state's perishable produce. These externalities include increased air pollution, damage to infrastructure (primarily pavement) and truck crashes that harm public safety. If California growers increased their use of rail, significant benefits could therefore accrue to the public. Such a shift might also improve the agriculture sector's resiliency amid natural disasters. Additionally, if the price of diesel continues to increase and turnover among long-haul truck drivers remains high, a shift toward rail could benefit growers economically. Though a transition from truck to rail transport would entail several challenges, such obstacles could likely be overcome through concerted effort by growers, buyers, public agencies and railroads.

Upward trend

California perishables, as shown in table 1, traveled over 2 billion ton-miles by rail in 2013. Among items shipped by rail, durable items such as oranges and carrots predominated. For carrots, 26% of the state's total production traveled by rail; for celery, onion and broccoli, the corresponding figures were 5.6%, 2.6% and 2%. (These figures were arrived at by dividing the tonnage of each commodity traveling by rail, as shown

A unit train of refrigerated cars filled with produce crosses the country.

Quiet-Silence/flickr.com

in table 1, by the state's total production of that commodity, derived from the state agricultural report for crop year 2013 [NASS 2015].)

In short, only a modest proportion of California's perishable produce travels by rail. But rail's importance may be on an upward trend. In the Central Valley, the railroad industry has made a significant effort to increase profits through transport of produce. Fresno, Tulare and Kern counties produce the majority of the state's orange crop and account for the majority of California's orange crop traveling by rail today. Figure 1 shows the percentage of oranges from these three counties that was transported by rail from 2005 to 2013; between 2007 and 2011, the percentage climbed substantially, to over 9% from under 2%.

Why the increase? Likely because Railex, a rail and logistics provider, opened a carload rail facility in Delano (Kern County) in 2008. A carload is a full boxcar, approximately equivalent to 2.5 truckloads; the Delano facility was designed specifically for perishables. From Delano, "unit trains" composed exclusively of refrigerated boxcars (or "reefers") travel to New York in a guaranteed seven to eight days, a schedule competitive with trucking. The boxcars comprising these trains are typically filled at the Delano facility, but growers can also place their produce in refrigerated containers as soon as it is harvested. The containers can then be driven to a container ramp and loaded by crane onto a conventional container train or attached to a unit train dedicated to perishables. (In 2017, demonstrating renewed interest in the perishable produce market, Union Pacific purchased Railex and the Delano facility.)

TABLE 1. Top perishable produce commodities traveling by rail in 2013

Commodity	Average distance traveled*	Tons	Ton-miles
	miles		*millions*
Carrots	2,410	244,132	589
Fresh vegetables, unclassified	2,482	224,160	556
Oranges	2,466	151,100	373
Potatoes	2,518	47,504	120
Celery	2,403	47,096	113
Cantaloupes and melons	2,500	41,648	104
Citrus, unclassified	2,437	34,160	83
Onions	2,336	23,672	55
Edible nuts in the shell	2,765	22,080	61
Broccoli	2,440	20,400	50
Total	**2,458**	**855,950**	**2,104**

* Average distance weighted by tonnage.
Source: Surface Transportation Board 2015.

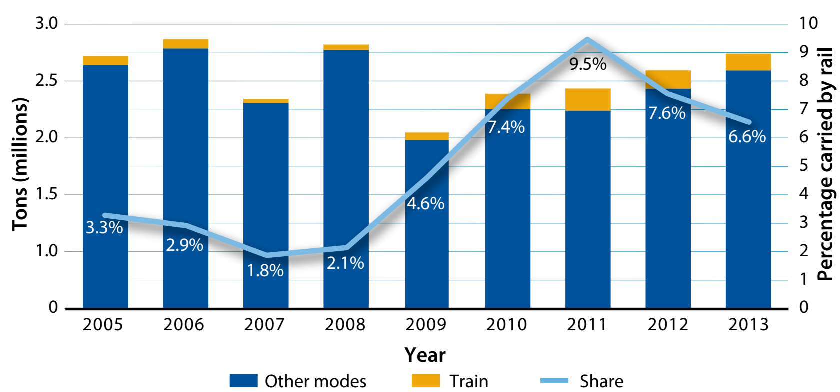


FIG. 1. Tonnage leaving California by rail — oranges. Source: Surface Transportation Board 2015.

Potential benefits

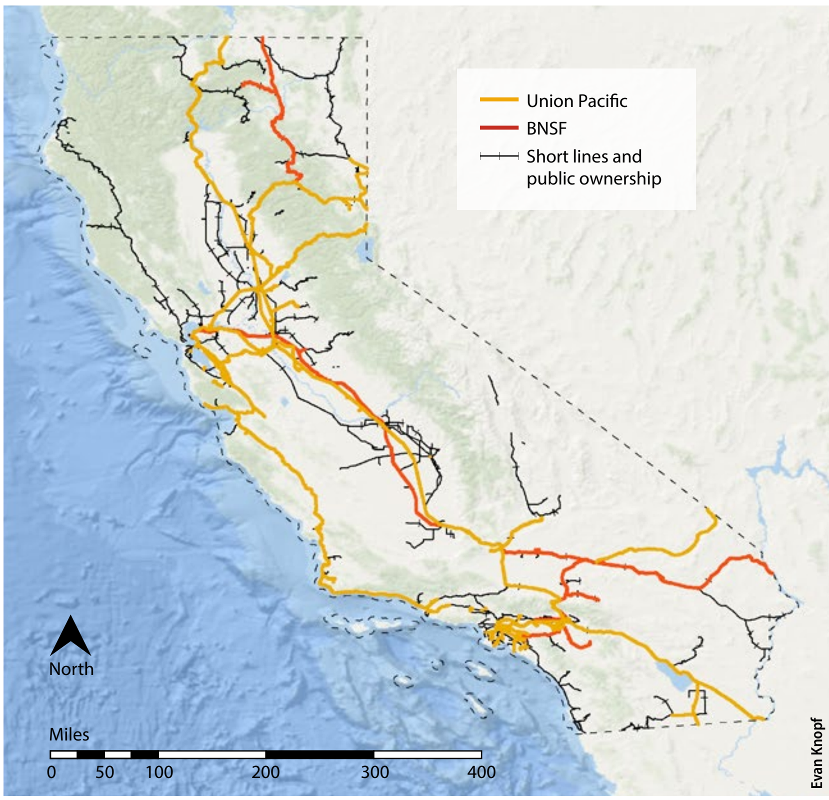
As part of research conducted for the Caltrans Division of Rail and Mass Transportation during 2015 and 2016, we examined peer-reviewed research that assessed various effects of rail travel as compared to truck travel (Seeherman and Hansen 2015). Working from these assessments, and calculating on the basis of the 2.1 billion ton-miles that California's perishable produce exports traveled by rail in 2013, we estimated that rail travel saved the public approximately $19 million by reducing four negative impacts: pavement damage, greenhouse-gas emissions, other polluting emissions and crashes.

To estimate these savings, we utilized existing life-cycle assessment analyses by Nahlik et al. (2015) and Facanha and Horvath (2007). These authors found that, due to the fuel efficiencies of rail as compared to trucks, greenhouse-gas emissions associated with freight trains were 0.44 pound lower per ton-mile than emissions associated with trucks (0.11 pound compared to 0.55 pound per ton-mile). We multiplied this difference by 2.1 billion ton-miles, resulting in a savings of roughly 900 million pounds of greenhouse-gas emissions. Taking into account current prices for carbon credits on the California cap-and-trade market ($14–$15 per metric ton, or about 2,200 pounds), this reduction in greenhouse-gas emissions equates to a savings in excess of $5.8 million. We performed similar analyses for the other three categories. The savings were $1.8 million for reduced pavement damage, $1.25 million for health care savings related to reduced air pollution and $10.4 million for crash reduction. Adding these to the $5.8 million saved due to reduced emissions of greenhouse gases, the total estimated annual savings are $19.25 million, or about $0.01 per ton-mile. (Readers are invited to examine the technical report on the rail transport of perishable produce for more detailed calculations related to the non–greenhouse gas categories [Seeherman and Hansen 2015].)

This estimate considers only one year. It considers only the small fraction of California perishables that already travels by rail. Therefore, the scope for additional savings could be significant. In 2017, to take one example, Monterey County harvested enough broccoli — 425,000 tons — to fill 17,000 trucks with 50,000 pounds of broccoli each (Monterey County 2017). This is an indication that increased transportation of perishable cargo by rail instead of truck could produce significant benefits both for infrastructure and for public health.

Barriers to overcome

A number of challenges stand in the way of achieving a meaningful shift from truck-based to rail-based transport of perishable produce. Though increased rail transport would yield savings for society, such savings can be challenging to visualize and would be difficult for growers to monetize. Perceived barriers include the current design of pallets, which are geared toward trucks and not trains; damage that produce can suffer due to the stronger vibrations involved in rail travel; spoilage resulting from travel delays; and a lack of needed infrastructure at rail terminals, particularly cold storage. But such barriers have sometimes been overcome in practice.



California is served by over 4,000 miles of track operated by the two long-haul interstate freight railroads, Union Pacific and BNSF, as well as an additional 800 miles operated by short-line railroads or public agencies.

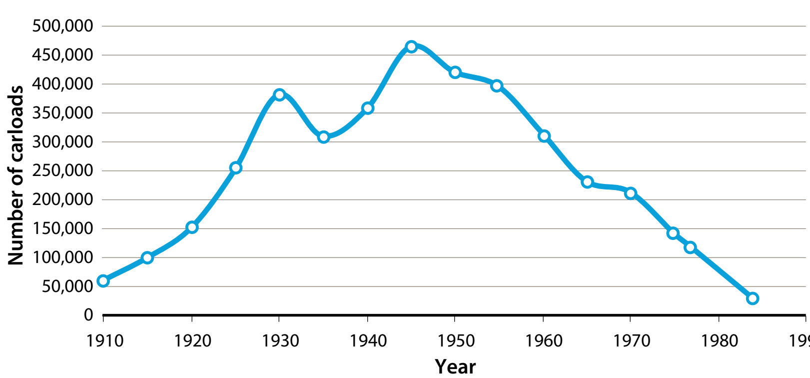


FIG. 2. Carload shipments by Pacific Fruit Express (PFE), 1910–1984. The steep decline in rail transport of produce from 1960 onward is well represented by the number of carloads (full boxcars, roughly equivalent to 2.5 truckloads) of produce moved by PFE, once among the nation's largest shippers of produce by rail. PFE was dissolved by its parent company in 1984. Source: Thompson et al. 2000.

When growers contemplate transporting their produce via rail boxcars, they sometimes harbor concerns about pallets and empty space within the cars. Such concerns can be alleviated through the use of domestic intermodal containers — rail containers equal in size to regular truck trailers, and transferable between the two modes of transportation. Refrigerated intermodal containers can be fully loaded at growing sites before being driven to a rail terminal.

Certain types of produce — fairly durable items such as root vegetables (carrots, potatoes, onions) and citrus — have increasingly been shipped by rail in the last several years. Specific types of green vegetables, such as broccoli, can also tolerate the vibrations associated with rail and are potential candidates for future increases in rail transport. Indeed, another fragile but much more lucrative commodity — wine — has recently experienced significant growth in rail transport. Between 2003 and 2013, the amount of California wine transported by rail increased by about 30%, to 1.8 from 1.4 million tons. Though boxed wine from San Joaquin County accounted for much of that increase, rail transport from Napa County significantly increased as well. This trend was advanced by a major grower, Kendall-Jackson, which built a terminal for its rail shipments. In 2013, nearly half of all wine exports, by weight, left California on a train (Ball et al. 2015).

Another key concern regarding shipment of perishable produce involves time to market and the possibility of spoilage. Some delicate exports — such as berries, whole tomatoes and bagged salad — cannot tolerate delays during transport or interruptions in refrigeration. However, because of Union Pacific's new time guarantees for its Delano reefer unit train, many types of durable perishables — for example, carrots, citrus and broccoli — can now be safely transported by rail.

Some local governments are examining the potential benefits of increased capacity for cold rail storage of perishable produce. For example, the Association of Monterey Bay Area Governments (AMBAG) has worked with local growers and Union Pacific to evaluate the construction of an intermodal terminal and associated cold storage facility just south of Salinas (AMBAG 2011). Monterey County is one of the most productive fruit and vegetable counties in the United States but exports virtually all of its produce by truck. AMBAG's report regarding cold storage near Salinas presented two key findings:

1. Intermodal rail represents a transportation option that can help the local produce industry remain

competitive — specifically by helping growers maintain relationships with wholesalers and distributors in an era when higher fuel costs and high turnover among long-haul truck drivers pose challenges for transporting produce by truck.

2. If increased use of rail transport removes from the Salinas Valley a portion of the trucks that now move produce out of the region, significant reductions in carbon emissions and air pollution will be realized.

The report estimated that if a cold storage facility were built near Salinas, demand would equate to about 180 to 200 domestic reefer containers per day, representing a small but significant fraction of the overall tonnage of produce exported from the county. The report estimated that use of reefer containers on this scale would eliminate the need for 46,800 full trucks per year; if each of those trucks is driven an average of approximately 3,000 miles, they collectively travel about 140 million miles. The report also found that switching from trucks to intermodal rail would not harm certain products currently transported by truck, notably broccoli and iceberg lettuce.

The report's authors, after examining truck and Union Pacific rate schedules, cited a transport savings to the East Coast of 5%–10%. The cost of building a dedicated intermodal ramp with cold storage in the Salinas Valley was estimated to be $20 million. The report's authors concluded that "This is the right time to move forward with the use of rail for the shipment of agricultural products from the Salinas Valley region." Union Pacific reported that it was willing to move this new cargo. The company had capacity available on its route along the California coast, and major railroads are attempting to diversify their portfolios because revenues from coal transport are undergoing a long-term decline. Thus, for Union Pacific, perishable produce was attractive as a potential new commodity. The project lost significant momentum when the price of diesel dropped in 2013 — but with prices now inching back toward $4 per gallon, growers may again push for modes of transport more efficient than trucking. (Both trucking and rail travel are primarily powered by diesel fuel, but because rail uses fuel more efficiently than trucking, rail travel becomes comparatively more attractive as diesel prices rise.)

Compelling argument

From 1960 to 1990, the vast majority of perishable produce transported out of California shifted from rail reefer cars to reefer trucks. Many of the factors behind this switch, such as differences in labor costs (due in part to higher rates of unionization among rail workers than truckers), persist to this day. Furthermore, after nearly 30 years of truck dominance, the forces of inertia make it challenging to reconfigure existing transport networks. Nevertheless, a compelling argument exists for moving at least some perishable produce back to rail. Rail boxcars and intermodal containers both exhibit a lower emissions profile than trucks; use of either will reduce traffic accidents and damage to freeway pavement; and both offer the flexibility of using a truck at either end of the rail journey. Rail transport of more durable produce types, such as oranges and root vegetables, has already proven successful, so a market for perishables already exists within the carload model (as shown in figure 1). Further growth in rail transport is likely to come from the intermodal market, which can accommodate a majority of produce types, including many green vegetables. As further noted by the AMBAG report, using modes of transport other than trucks could also improve resiliency in a disaster and increase the reliability of transport costs.

Given the obstacles to increased use of rail transport discussed above, a combined effort by railroads, growers, buyers and public agencies will be needed if movement of perishables is to transition from truck to rail. If diesel prices continue to increase and railroads achieve improvements in reliability and speed, rail could gain a greater share of the transport of perishable produce — a change that would most certainly reduce negative externalities and therefore benefit the public. CA

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COLLABORATIONS

4-H in the Outdoors: Delivering environmental education to Latino youth in Riverside County

4-H teams up with Project Learning Tree, and kids are the winners.

It's a bit of a paradox: Polling indicates that most Latinos in the United States place a high value on environmental conservation (Earthjustice 2015). But Latinos — especially in the younger age brackets — tend to participate in outdoor recreation at lower rates than members of other demographic groups (Outdoor Foundation 2017).

It's an imbalance that Claudia Diaz Carrasco decided to do something about.

This is my first time touching the river. I love it! It's so cold!—*Natalie (five years old)*

Diaz, a 4-H youth development advisor, recognized a real need around her home turf of Riverside and San Bernardino counties to provide urban Latino youth with environmental education. But she faced a serious obstacle. Diaz and her 4-H colleagues, she says, are "experts on youth development — not environmental education."

Enter Sandra Derby, the UC ANR–based California coordinator for Project Learning Tree — an international organization that, according to Derby, aims for students "to get outdoors, connect with their own environment, become the drivers of their own learning." Project Learning Tree develops a rich variety of educational curricula, but it doesn't deliver them to students directly. Rather, it trains outside educators, who in turn guide students through Project Learning Tree programs.

If it sounds like a good fit, it was, and collaboration between 4-H and Project Learning Tree soon resulted in a program called 4-H in the Outdoors. "The program was marked for success," Derby says.

The basic mission of 4-H in the Outdoors is to prepare youth in the program to appreciate and safely explore the world outside. In a fairly typical example of the project's work, Stephanie Barrett — a 4-H program representative in Riverside County — led fourth-grade classes at Little Lake Elementary through a process of tree-focused discovery. The youngsters studied tree rings. They "adopted" a tree on school grounds, observing and writing about it. They imagined — and rejected — a world without plants. On a field trip to the nearby Idyllwild Nature Center, they even braved a nature hike. "The program," Barrett says, "is very hands-on and experimental. It asks kids to think about their own role in the environment. It teaches them how to think — not what to think."

In only its second full year of operation, 4-H in the Outdoors has reached more than 2,000 children. "We're really busy," Barrett says, adding without evident chagrin that "I got home at 11 p.m. two nights this week."

4-H in the Outdoors has received grants from organizations such as the Southern California Environmental Education Collaborative. Local partners have provided free bus transportation and free admission to nature areas. Mostly, though, the program has benefited from hard work — and from natural synergies between two programs housed within UC ANR.

Project Learning Tree is funded in California by Cal Fire, with support from forestry-based groups such as the Sustainable Forestry Initiative, the parent organization of Project Learning Tree. More information about Project Learning Tree's programs and materials can be found at www.plt.org or ucanr.edu/sites/PLT_UCCE. CA

—*Lucien Crowder*

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NEWS

Coordinated response to inadvertent introduction of pathogens to California restoration areas

Growers, regulators and native plant restoration experts are together trying to reduce the spread of more than 25 new *Phytophthora* taxa

Susan J. Frankel, Plant Pathologist, Ecosystem Function and Health, USDA Forest Service, Pacific Southwest Research Station

Janice M. Alexander, Forest Health Program Coordinator, UC Cooperative Extension Marin County

Diana Benner, Owner, Watershed Nursery

Alisa Shor, Director of Park Nurseries, Golden Gate National Parks Conservancy

Phytophthora tentaculata was cultured from the roots of this nearly dead sticky monkey flower (*Diplacus aurantiacus*) 2 years after outplanting in a restoration area in Alameda County.

In 2012, a plant pathogen known as *Phytophthora tentaculata* was discovered on sticky monkey flower (*Diplacus aurantiacus*) at a native plant nursery in Monterey County (Rooney-Latham and Blomquist 2014). Subsequently — in a development that stunned restoration ecologists as well as growers at native plant nurseries — evidence emerged that *P. tentaculata*, *P. cactorum* and other new or new hybrid *Phytophthora* species had been unintentionally but extensively introduced into restoration areas in the greater San Francisco Bay Area (Garbelotto et al. 2018, page 208 in this issue). These soilborne plant pathogens, as they move from native plant nurseries to restoration sites via planting stock, potentially undermine the very purpose of restoration projects. That is, they degrade rather than enhance habitat — not only causing plant mortality but also threatening ecological investments intended to improve habitat for vulnerable species such as coyote ceanothus (*Ceanothus ferrisiae*), California tiger salamanders (*Ambystoma californiense*), California red-legged frogs (*Rana draytonii*) and mission blue butterflies (*Aricia icarioides missionensis*).

In 2015, in an effort to protect sensitive habitats and restoration sites against *Phytophthora* and other introduced plant pathogens, we created an organization called the Phytophthoras in Native Habitats Work Group and invited any interested parties to join. The Work Group's aim is to minimize the spread of *Phytophthora* pathogens in restoration sites and native plant nurseries by coordinating a comprehensive, unified program entailing management, monitoring, research, education and policy. The Work Group — modeled after the California Oak Mortality Task Force — builds consensus; provides technical assistance to stakeholders such as government agencies, nursery growers and nonprofit organizations; develops strategies and techniques to support adaptive integrated pest management programs to address *Phytophthora* species in restoration areas; and identifies funding needs and available resources to protect wildlands and assist the restoration industry in its efforts to contain *Phytophthora*.

The problem

When *P. tentaculata* was discovered in Monterey County in 2012, it was the first time the pathogen had been detected in the United States. *P. tentaculata* was subsequently detected on toyon (*Heteromeles arbutifolia*), coffeeberry (*Frangula californica*) and sage (*Salvia* spp.) in nurseries and at restoration sites in several California counties (Rooney-Latham et al. 2015). Further investigations detected *P. quercina* — a microbe with high potential to damage U.S. environmental and economic interests (Swiecki and Bernhardt 2017) — on a planted valley oak (*Quercus lobata*) in a restoration area in San Jose (Santa Clara County). As with *P. tentaculata*, this was the first time *P. quercina* had been detected in the United States. A survey of native plant nurseries and restoration areas, conducted from 2014 through 2016, identified well over 25 *Phytophthora* taxa in the nurseries or in plantings that originated from the nurseries (Rooney-Latham et al. 2017), along with at least 70 new associations between *Phytophthora* and native plant species.

Two decades earlier, the sudden oak death pathogen (*P. ramorum*) had emerged from nurseries to create an uncontrollable epidemic of wildland plant disease. Now, detections of *Phytophthora* sparked concern that, once again, invasive species could travel the nursery-to-wildland pathway to cause an inadvertent but uncontrollable epidemic. The sudden oak death pathogen had been introduced to California and Oregon on ornamental nursery stock, most likely rhododendrons or camellias. It then moved via windblown rain into adjacent forests, taking hold in several areas along the Pacific coast and killing millions of tanoaks (*Notholithocarpus densiflorus*) and coast live oaks (*Quercus agrifolia*) (Rizzo et al. 2002). The spread of the oak pathogen demonstrated how, when container plants are transported, pathogens such as *Phytophthora* can travel long distances and proliferate across landscapes (Croucher et al. 2013; Goss et al. 2009; Grünwald et al. 2012). Once an area is contaminated, it is difficult or impossible to eradicate the pathogen and to restore lands (Goheen et. al 2017; Kanaskie et al. 2017).

The response

Some land managers, in response to soilborne *Phytophthora* introductions, suspended plantings, cancelled orders of nursery stock or invested millions of dollars in solarization treatments to clean up contaminated sites. But such measures achieved only partial eradication (Hillman et al. 2017; Lyman et al. 2017) — and in any event, neither discontinuation of restoration planting nor switching to direct seeding represents an ideal long-term approach to *Phytophthora* prevention. Many benefits of restoration are foregone or significantly delayed when nursery stock is not used.

The Work Group has pursued a collaborative approach to protecting native plant habitats. Participants — growers of native plants, vegetation managers connected with water districts and open space areas, restoration consultants, plant pathologists, plant health regulators and environmental regulators — have established an interdisciplinary process for developing best management practices regarding production at restoration nurseries, planting at restoration areas and maintenance of sites. The group has produced guidance that can help environmental regulators reduce the risk of *Phytophthora* spread (the guidance involves altering certain elements of restoration design and instituting changes to regulators' criteria for success). The group also has organized a number of symposia and trainings and has posted key *Phytophthora* information on the internet

Phytophthora cinnamomi introduced into native habitats can kill susceptible species, such as the giant chinquapin (*Chrysolepis chrysophylla*) visible in this photo taken in the Oakland Hills (Alameda County). Pallid manzanita (*Arctostaphylos pallida*) mortality due to *P. cinnamomi* infection is also present in this stand.

Ted Swiecki, Phytosphere Research

(calphytos.org). Partly in response to the group's work, several prominent managers of restoration nurseries proactively redesigned their facilities, imposing strict phytosanitary measures to prevent *Phytophthora* spread; they then shared stories of their success in growing healthier plants, inspiring other nursery managers to create clean areas for stock production (Sims et al. 2018). Organizations — with the help of interns, staff or contracted workers — sampled their properties to better understand the incidence and distribution of pathogens. Regulators such as the Army Corps of Engineers are incorporating Work Group guidance into environmental assessments. Finally, researchers are actively working on novel diagnostic approaches, including the use of dogs to sniff out pathogens (Swiecki et al. 2018, page 217 in this issue).

Concurrently, the California Native Plant Society adopted a policy intended to prevent harmful pathogens from spreading out of native plant nurseries or spreading via plant sales by the society's chapters; the policy fosters the use of clean, native plant stock in all landscape and restoration plantings. In February 2017, State Senator Bill Dodd introduced SB 287, entitled "Habitat restoration: invasive species: *Phytophthora* pathogens," which would require the California Department of Fish and Wildlife to adopt regulations to minimize the risk associated with *Phytophthora* pathogens in plant materials used for habitat restoration projects authorized, funded or required by the state. The Work Group, to simplify compliance with and enforcement of the prospective regulations, is exploring a program for restoration nursery accreditation or certification, which could be incorporated into the bill. Meanwhile, the California Department of Food and Agriculture revised its pest risk ratings for several *Phytophthora* species; the new ratings enable county agricultural commissioners to take control actions if they deem them warranted.

More to do

Many native plant nurseries, by adopting systematic phytosanitary measures, have committed themselves to preventing pathogen introductions. Much work remains to be done before all restoration nurseries are operating at the highest standards, but we are heartened by the progress made thus far and by the coalition's enthusiasm about the work still to come. Potential sources of contamination, however, are not limited to native plant nurseries — nurseries of all types pose risks. Many experts therefore agree that risks surrounding *Phytophthora* species and other plant pathogens (Swiecki and Bernhardt 2017) should be factored into hazard assessments for all nursery stock. Restoration nurseries have implemented phytosanitary measures to protect the diversity and vitality of natural vegetation — but because interconnections between people and nature are forever increasing, such measures might deserve wider adoption. CA

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REVIEW ARTICLE

Soil- and waterborne *Phytophthora* species linked to recent outbreaks in Northern California restoration sites

A review identifies several *Phytophthora* species found in California wildlands and discusses approaches for preventing and diagnosing the spread of these plant pathogens.

by Matteo Garbelotto, Susan J. Frankel and Bruno Scanu

Abstract

Many studies around the globe have identified plant production facilities as major sources of plant pathogens that may be released in the wild, with significant consequences for the health and integrity of natural ecosystems. Recently, a large number of soilborne and waterborne species belonging to the plant pathogenic genus *Phytophthora* have been identified for the first time in California native plant production facilities, including those focused on the production of plant stock used in ecological restoration efforts. Additionally, the same *Phytophthora* species present in production facilities have often been identified in failing restoration projects, further endangering plant species already threatened or endangered. To our knowledge, the identification of *Phytophthora* species in restoration areas and in plant production facilities that produce plant stock for restoration projects is a novel discovery that finds many land managers unprepared, due to a lack of previous experience with these pathogens. This review summarizes some of the key knowledge about the genus *Phytophthora* in general and lists some of the many soilborne and waterborne species recently recovered from some California restoration sites and plant production facilities.

Historically, the release of *Phytophthora* species in the wild has resulted in massive die-offs of important native plant species, with cascading consequences on the health and productivity of affected ecosystems (Brasier et al. 2004; Hansen 2000; Jung 2009; Lowe 2000; Rizzo and Garbelotto 2003; Swiecki et al. 2003; Weste and Marks 1987). Once introduced, plant pathogens in general cannot be eradicated (Cunniffe et al. 2016; Garbelotto 2008), and costs associated with the spread and control of exotic pathogens and pests have been estimated to surpass $100 billion per year for the United States alone (Pimentel et al. 2005). Thus, preventing the introduction of pathogens by using pathogen-free plant stock is the most cost-effective and responsible approach (Parnell et al. 2017).

In their extensive meta-analysis, Santini et al. (2013) identify the trade of live plants as the main pathway for the introduction of invasive forest diseases in Europe. Similarly, Jung et al. (2016) identified plant production facilities as a major source of

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Matteo Garbelotto

Phytophthora diseases are increasingly being found in California wildlands and parks, where they have caused large die-offs of native plant species. Shown here is Ione manzanita (*Arctostaphylos myrtifolia*) in Ione, California, killed by *P. cinnamomi.*

Phytophthora inoculum that may be released in the wild. The best-known example of a *Phytophthora* species released in California natural environments from commercially produced plants is that of *Phytophthora ramorum* (Grünwald et al. 2012), but an equally important prior introduction associated with infested plant nurseries is that of *Phytophthora lateralis*, which affected Port Orford cedar in California and Oregon (Hansen et al. 2000).

Recently, Rooney-Latham and colleagues (Rooney-Latham and Blomquist 2014; Rooney-Latham et al. 2015) identified at least two soilborne *Phytophthora* species, including one reported for the first time ever in the United States, as the cause of extensive mortality of two plant species recently employed in an extensive restoration project. Both species were also found in the production facilities that had supplied the plant stock, and both species have been shown, through greenhouse inoculation studies, to be aggressive pathogens on three important hosts present in the restoration areas (Sims et al. 2018). This discovery triggered multiple surveys of failed restoration projects and of the facilities that provided plants employed in such projects (Frankel et al. 2018). While soilborne and waterborne Phytophthoras have been found in commercial production of orchard and landscaping plants, to our knowledge this is the first reported case of *Phytophthora* species found in plants bound for native landscapes (Frankel et al. 2018; M. Garbelotto, unpublished results). Although *Phytophthora* species are known to be plentiful in commercial plant production facilities, their discovery in native plant production facilities is novel, and finds many land managers unprepared, due to a lack of previous experience with these pathogens.

Given that the research community has been focused on aerial *Phytophthora* species such as *P. ramorum* recently, this review summarizes some basic knowledge for soilborne and waterborne *Phytophthora* species, such as those recently recovered from restoration and disturbed sites in the San Francisco Bay Area in California. Even if we acknowledge that infected plants can often be asymptomatic (Bienapfl and Balci 2014; Jung et al. 2016; Migliorini et al. 2015), we hope this article may increase the awareness about this group of pathogens, possibly leading to their early detection in plant production facilities (Parke et al. 2014; Patel et al. 2016), before infected plants are outplanted in the wild.

Introduction to the genus *Phytophthora*

For decades, *Phytophthora* species have been erroneously lumped with the Fungi, but in order to fully understand their biology and ecology it is important to understand their correct taxonomic position. The genus *Phytophthora* belongs to the kingdom Straminipila (formerly Chromista), which also includes aquatic organisms such as diatoms and kelp (Dick 2001). The genus *Phytophthora* is part of the order Peronosporales: this order contains genera that are notable for having co-evolved with plant hosts mostly as plant pathogens, although some are pathogens of animals (Spies et al. 2016; Thines 2014). The four best-known genera are *Peronospora*, *Plasmopara*, *Pythium* and *Phytophthora*. Each has evolved distinct epidemiological strategies. While *Peronospora* and *Plasmopara* species (causal agents of plant diseases known as "downy mildews") mostly spread aerially, *Pythium* species are almost exclusively soilborne and waterborne. The genus *Phytophthora* stands between the two, and includes species that are soilborne and waterborne, or airborne, and some species with a mixed epidemiological strategy (Bourret et al. 2018; Oßwald et al. 2014).

Phytophthora propagules responsible for much of the known host-to-host spread are normally ovoid or pyriform in shape and are called sporangia (fig. 1A). Sporangia can be extremely variable in form and size and are normally produced alone or in clusters at the end of stalks. If sporangia can be easily detached from the stalks that bear them, the species may be aerially dispersed rather than just being soilborne and/or waterborne (Erwin and Ribeiro 1996).

Sporangia of all *Phytophthora* species, when mature, contain a variable number of motile, biflagellate zoospores (fig. 1B). Sporangia sometimes can germinate directly and infect a plant, or plants can be infected directly by hyphae growing in the soil. However, it is the zoospores that are mostly responsible for infection of plant tissue. Zoospores are normally attracted by chemical or electrical signals generated by the plant host (Carlile 1983) and require a film of water to "swim" and initiate the infection process. If there is no film of water or water dries out, zoospores can encyst and become dormant without losing viability. Infection by zoospores or by germinating sporangia can occur through stomatal openings, or an infection peg can rupture the plant cell wall and directly infect plant tissue (Erwin and Ribeiro 1996). The need for a film of water for zoospore-mediated infection to occur largely explains the direct relationship between increasing disease levels and increasing rainfall values.

Phytophthora species also produce spherical survival structures called chlamydospores (fig. 1C). The size of chlamydospores, the pattern and the abundance in which they are produced, and the thickness of their outer wall can often be diagnostic traits differentiating *Phytophthora* species. Chlamydospores can survive up to several years in adverse environmental conditions; they can also contaminate soil and water and be responsible for dispersal of the pathogen. In favorable conditions, chlamydospores can germinate directly or they can produce a sporangium. Like sporangia, chlamydospores are clonally produced and do not require mating.

Sexual structures produced by *Phytophthora* species after mating are called oospores and are produced by a single individual in homothallic species, or when two individuals bearing different mating types come into contact in heterothallic species. Exposure of heterothallic species to certain fungi or chemicals can also trigger the formation of oospores in the absence of mating (Pratt et al. 1972; Uchida and Aragaki 1980). Oospores are particularly thick walled and can also be regarded as long-term survival structures, often even more resilient to adverse conditions than chlamydospores (fig. 1D). Note that oospores of homothallic species will be genetically identical to the individual that produced them, because recombination between homologous chromosomes cannot generate variation, while oospores of heterothallic species will be genetically different from the two parents. Sexually generated variation may help the pathogen to adapt to novel environments or hosts.

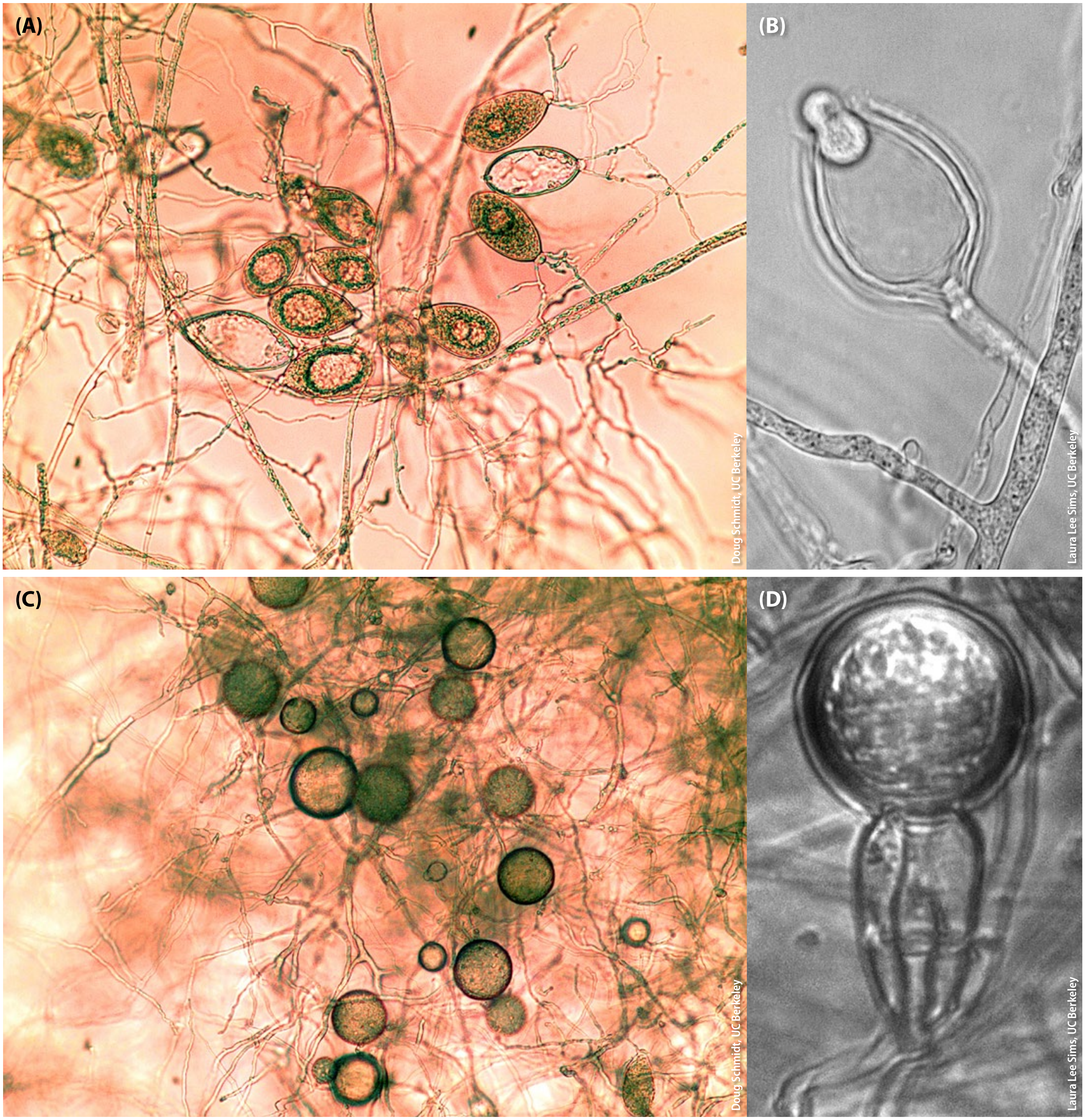


FIG. 1. Micrographs (300× magnification) of (A) sporangia of *Phytophthora ramorum*, (B) a zoospore exiting a sporangium of *Phytophthora bilorbang*, (C) chlamydospores of *Phytophthora ramorum* and (D) an oospore of *Phytophthora alni* subspecies *uniformis*.

In addition to variation in morphological traits among different species, *Phytophthora* species have been differentiated based on the traits listed below. Some of these traits may have important implications for disease management and modeling (Erwin and Ribeiro 1996). For instance, one may assume that the release of a "cold-weather" *Phytophthora* species in a warm region may be relatively unsuccessful:

(1) Temperature preferences: that is, adaptation to warm, cool or cold environments (Cooke et al. 2000).

(2) Ability to infect a large number of unrelated hosts (generalists) versus ability to infect only closely related or a limited number of hosts (specialists) (Oßwald et al. 2014).

(3) Mode of reproduction. Individuals belonging to homothallic species can complete the sexual stage and produce oospores without mating. Two individuals carrying opposite mating types (namely A1 and A2) are needed instead by outcrossing, heterothallic species. It should be pointed out that sporangia are produced asexually both in homothallic and heterothallic species, so normally lack of sex does not interfere with spread of a species. Also, it seems plausible that homothallic species may survive in harsher climates (M. Garbelotto, unpublished data), thanks to the fact they can often easily produce oospores without the need for mating with a compatible strain.

(4) Range of soil pH preferred for growth (Kong et al. 2009).

(5) Evolutionary relationship or relatedness. Species belonging to the same clade (a clade is a group of closely related species that evolved from the same ancestor; based on Jung et al. [2017] there are at least 12 clades in the *Phytophthora* genus) often have similar biology and can hybridize (Brasier et al. 2004; Husson et al. 2015). Hybrids, however, may differ in host range and virulence from the parental species.

(6) Virulence. Some *Phytophthora* species may be defined as opportunistic, requiring a weakened host for infection or colonization, while other species are aggressive primary pathogens, leading to severe symptoms, impairment or mortality independent of host health status (Jung et al. 2011). This distinction is key in predicting the impact of emergent *Phytophthora* species; however, it is variable and the virulence of a species may change due to variation in the host or in the environment.

(7) Aerially spreading, or spreading through infested soil or water (Scanu and Webber 2016).

Soilborne and waterborne versus aerial species

The part of the plant that a *Phytophthora* species infects (roots, foliage or stem) drives many aspects of disease epidemiology. It is unclear what makes a *Phytophthora* species well adapted to be either airborne and primarily infect aerial parts of plants, or to be soilborne or waterborne with infections primarily limited to the roots and root collars. In the second case, aboveground symptoms are not caused directly by infection but are a consequence of root mortality and of girdling of the root collar (fig. 2). It should be noted that the distinction between airborne and soilborne or waterborne species is not always clear-cut. In general, we define as airborne those species that spread through

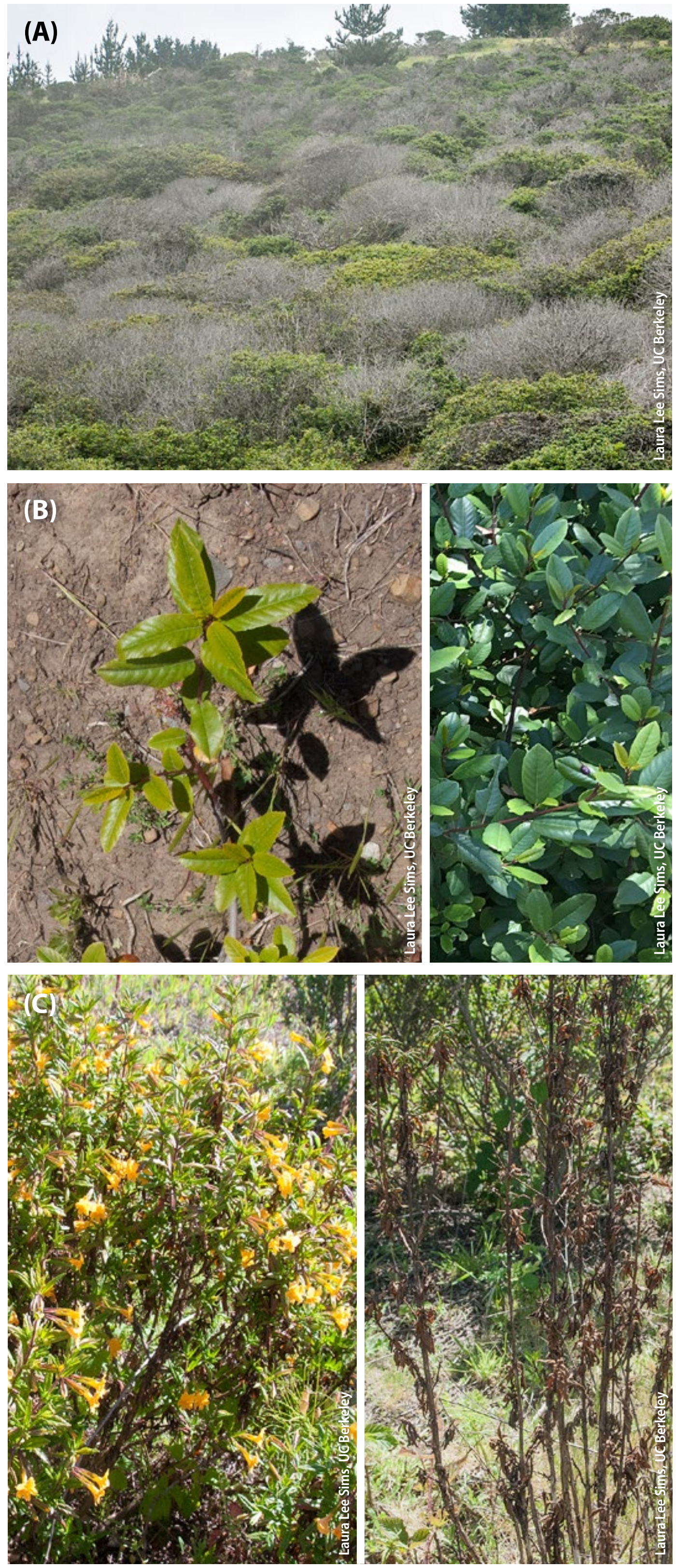


FIG. 2. Visible symptoms caused by root and root collar infection by soilborne and waterborne *Phytophthora* species. (A) Coffeeberry (*Frangula californica*) in San Mateo County infected by *Phytophthora multivora*; (B) coffeeberry outplanted in Marin County infected by *Phytophthora megasperma* on the left, and healthy coffeeberry on the right; and (C) healthy sticky monkey flower (*Diplacus aurantiacus*) on the left, and plants infected by *Phytophthora megasperma* on the right.

airborne propagules, while the soilborne and waterborne category includes species that mostly spread through soil and water contaminated by propagules. To be more precise, some species within the soilborne and waterborne group appear to be better adapted to live in water (e.g., lakes, streams, ponds), while others may preferentially be found in matrical soil water. However, we believe this difference to be often debatable and have decided to group together soilborne and waterborne species in the same group. Table 1 compares a few important traits between soilborne and waterborne and airborne species.

TABLE 1. A quick comparison of a few traits of soilborne/waterborne and airborne *Phytophthora* species

Soilborne and waterborne	Airborne
They infest soil and water, and mostly infect roots and root collar. They can also infect aerial portions of plants through infested tools or splash of soil or water particles (Madden et al. 1992; Scanu and Webber 2016; Trione and Roth 1957).	They can be found in soil and water, so infested soil and water can be responsible for their spread. Infections occur mostly on aerial plant parts, but occasional root infections are possible (Rizzo et al. 2005).
They can survive for relatively long periods in soil or potting media. Survival may be independent of plant debris present in the soil (Vettraino et al. 2010), while sporulation appears to be linked to the presence of roots or root fragments embedded in the soil (Jung et al. 2013).	They can survive in soil but are not extremely long-lived (Fichtner et al. 2007) and are less competitive than soilborne and waterborne species (Eyre et al. 2013). Conversely, survival in inert potting media can be extensive (Shishkoff 2007).
Production of chlamydospores, or oospores or stromata-like hyphal aggregations (masses of vegetative structures) may be necessary for long-term survival in soil (Crone et al. 2013).	Production of chlamydospores, or oospores or stromata-like hyphal aggregations (masses of vegetative structures) may be necessary for long-term survival in soil (Crone et al. 2013).
Sporangia can be caducous or not caducous (Erwin and Ribeiro 1996).	Sporangia are almost always caducous (i.e., deciduous) (Erwin and Ribeiro 1996).

A consequence of being soilborne or waterborne is an extremely patchy distribution at the landscape level. However, the distribution of soilborne or waterborne *Phytophthora* species can be further expanded through various human-related mechanisms, including planting of infected plants and movement of soil along roads or paths (Krull et al. 2013; Ristaino and Gumpertz 2000). Additionally, once introduced in a site, propagules of these pathogens will move on their own following gravity and movement of water in waterways and in underground water tables (Maurel et al. 2001). When humans are not directly involved in their spread, these pathogens often appear to move more easily downhill than uphill. Downhill spread can be significant because it occurs via both root contacts and downward movement of infested water or contaminated soil. Uphill movement, by contrast, is usually more limited, because it relies almost exclusively on root contacts.

There are some commonalities among all soilborne and waterborne species: They tend to be more abundant in soils with a loamy to clay structure and less abundant in sandy, well-drained soils (Cook and Papendick 1972); their frequency increases as rainfall and temperature increase (Thompson et al. 2014); and high levels of soil infestation are associated with soils that are poor in organic matter (Weste and Marks 1987), as in the case of serpentine soils (Shearer and Crane 2011). Furthermore, disease development appears to be more marked in those climates that alternate between wet and dry periods, for example, regions characterized by a Mediterranean climate (Burgess et al. 2016). The reasons behind marked disease severity in areas with Mediterranean climate may be twofold. First, wet-dry cycles maximize the frequency and the duration of periods in which soil is wet but not saturated at field capacity; in fact, anaerobiosis in saturated soils actually depresses sporulation by Phytophthoras (Nesbitt et al. 1979). Second, plants infected during wet periods may then become more susceptible to colonization by Phytophthoras due to the stress induced by prolonged periods of drought (Desprez-Loustau et al. 2006).

Establishment and spread of exotic species

Major pathways for the initial primary introduction of exotic soilborne and waterborne *Phytophthora* species in a new region include the use of infected plant material or of infested soil (Liebhold et al. 2012; Parke et al. 2014). *Phytophthora* inoculum (e.g., infectious propagules) may be present either in infected plant tissue, in the soil plants have been grown in, or in both (Jung et al. 2016). Once introduced in a new site, secondary spread up to a few meters per year can be the result of root-to-root infection or of infection of roots by hyphae, and of movement of infectious or survival structures (sporangia, chlamydospores and oospores) through splash (Ristaino and Gumpertz 2000), or of the movement of insects or small animals that may carry *Phytophthora* propagules on their bodies. Longer-range spread, up to tens or even hundreds of kilometers per year, can occur through soil movement due to vehicular traffic or to animal movement, and through the movement of infested water.

Spread through infested water may occur at different spatial scales: a few meters when dealing with matrical water (i.e., water present among soil particles), tens or hundreds of meters for runoff water, hundreds or even thousands of meters for infested underground water tables (Hayden et al. 2013), and even longer distances for infested water carried in streams and rivers as evidenced for the spread of *P. lateralis* in southern Oregon and Northern California (Hansen et al. 2000). Infested water can also be moved by helicopter or trucks used for firefighting or for road dust abatement. Spread at the landscape level is thus affected by abundance of roads and streams, by intensity of human activities, by topography (with draws and depressions being more conducive to spread), by abundance of favorable sites (clay soils, lower organic content) and by densities of animals and, especially, of susceptible hosts. Abundance of snails and ants may also contribute to increase disease severity in a site (El-Hamalawi and Menge 1996).

Increasing host diversity in a site may have diametrically different effects on disease spread rate and disease severity. When the percentage of infectious hosts increases (note that some hosts may be susceptible but not infectious), so do disease spread rate and disease severity. This is, for instance, the case of some *Lupinus* species present in woodlands infested by *P. cinnamomi* in Spain (Serrano et al. 2010). Conversely, when increased host diversity leads to a decrease of percentage of the more infectious hosts, an effect called "inoculum dilution" leads to decreased spread rates and disease severity (Haas et al. 2011).

Prevention and diagnostics of *Phytophthora* species

The most effective control of soilborne or waterborne Phytophthoras relies either on the prevention of their introduction or on slowing their further spread, once introduced. Prevention of primary introductions can be achieved by properly testing plant material to be outplanted and by using stock produced in facilities that observe best management practices (BMPs) aimed at limiting establishment of these soilborne pathogens in soil, pots and water systems as well as plants (Parke and Grünwald 2012). Recently, BMPs aimed at reducing risk of infestation have become available (see Sims et al. 2018 or www.suddenoakdeath.org/wp-content/uploads/2016/04/Restoration.Nsy_.Guidelines.final_.092216.pdf and http://ucanr.edu/phytophthorabmps).

Notwithstanding the use of material produced in facilities adhering to such BMPs, it has been repeatedly advised to place all new plant material in a quarantined area for several weeks and to observe it for the onset of symptoms (Alexander and Lee 2010). In the absence of a certificate indicating the production facility is free of *Phytophthora* species (Brasier 2008), a direct inspection of plants to be purchased needs to be performed, including observations of the health status of root systems.

Four different approaches may be utilized for direct testing of these substrates:

(1) Baiting. Plant material (symptomatic and asymptomatic), root and soil samples can be baited by submerging the sample in water and floating baits comprised of susceptible plant parts such as leaves and fruits. Baiting must be done under aerobic conditions assured by mixing the correct amounts of plant material or soil and water (see Erwin and Ribeiro 1996), but protocols vary greatly with regards to specific baiting protocols (Jung et al. 1996; Scanu et al. 2013). Different baits (e.g., consisting of different plant species or of different plant parts) may not be equally effective when trying to detect different *Phytophthora* species (Erwin and Ribeiro 1996). In some cases, drying the soil before baiting is recommended (Erwin and Ribeiro 1996). One advantage of baiting is that precise knowledge of the exact portion of the plant or the specific soil particles that may contain viable *Phytophthora* infection is not needed; for this reason, baiting is one of the preferred diagnostic approaches when surveying large facilities, soil and wildland waterways. However, for unknown reasons, some species are difficult to detect by baiting and thus negative baiting results can represent false negatives. Furthermore, baiting requires experience, particularly in the identification of the agent causing the symptoms on the bait, which can be done by direct culturing or by the use of molecular approaches on symptomatic tissue (see 2 and 3 below).

(2) Direct isolation from symptomatic (or asymptomatic) plant tissue using *Phytophthora* selective media (Jeffers and Martin 1986; Scanu et al. 2014). There are a few drawbacks of direct isolation: (a) one needs to sample a portion of the plant where the pathogen is viable and viability may be dependent on season and/or phenological state of the host plant; and (b) some species may have almost identical morphology and therefore are difficult to identify correctly without molecular testing. The most significant drawback of this approach is that sampling requires destructively excising a portion of the plant, and often that requires destructively manipulating plants to identify symptomatic portions to be plated. False negatives for both direct isolation and baiting techniques can occur in the case of species that are not easily culturable, or due to the presence of secondary microorganisms preventing Phytophthoras from growing axenically.

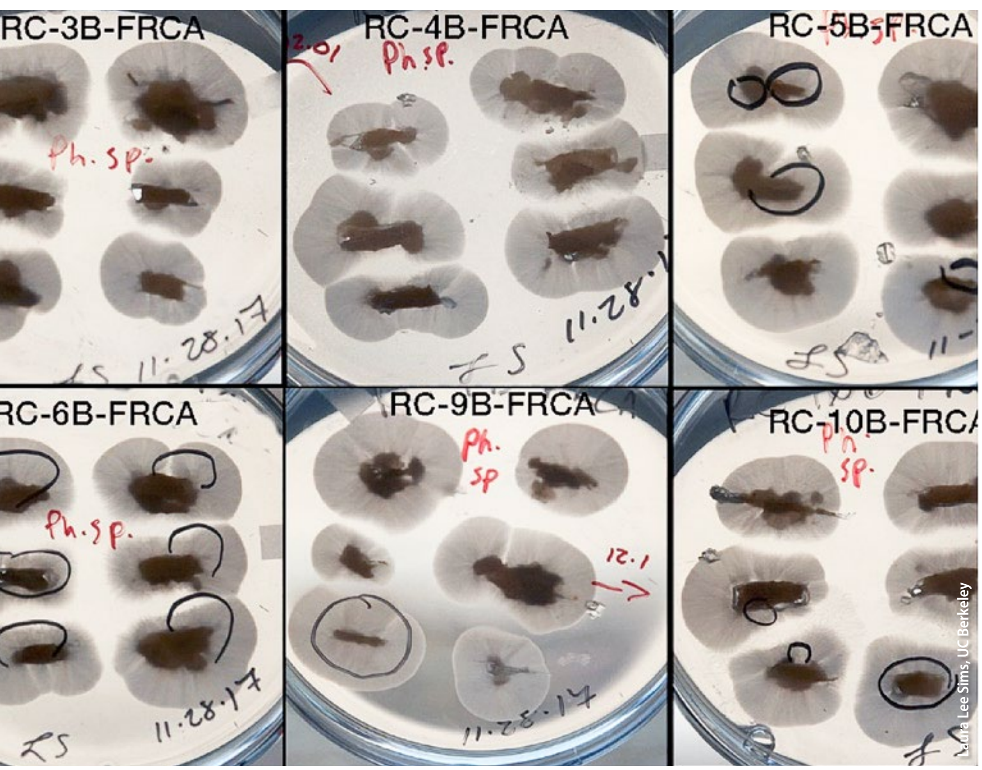
(3) Molecular identification techniques are based on the detection of specific sequences of nucleic acids (DNA, RNA) (Martin et al. 2012; Prigigallo et al. 2015). Molecular approaches are not dependent on the viability of the pathogen but do require that the correct portion of an infected plant be processed. Additionally, there are risks of false positives due to either lab contamination or to a lack of specificity of the assay detection probes, caused either by the existence of undiscovered closely related species or by poor probe design. False negatives are commonly caused by poor processing or by the presence of inhibitors, whose concentration in tissues or substrate may vary depending on time of year and material sampled.

The high sensitivity of molecular approaches thus can be regarded both as a benefit and a drawback. A benefit because it allows the detection of relatively young incipient infections or infections in remission characterized by a low amount of pathogen DNA (Hayden et al. 2004). A drawback because results with such approaches may not be informative as to the viability of the pathogen, due to the fact that unviable dead cells of the target organism may also be detected (Chimento et al. 2011).

Molecular identification assays normally are based on one of two approaches: (a) Results may be positive or negative and based on the success or failure of assays specifically designed to target one or a few species. Or (b) Results may be based on the homology (e.g., similarity) of DNA sequences of so-called barcode genetic loci. The two most common barcode loci for *Phytophthora* species identification are the nuclear internal transcribed spacer (ITS) and the mitochondrial cytochrome oxidase (COX) (Cooke et al. 2000; Martin et al. 2014). In general, homology has to be 98% or higher between a published sequence and the sequence of an unknown sample to identify the unknown. Most conspecific genotypes have a DNA homology of 99% to 100%. Sequences are published in several databases, but the most commonly used one remains GenBank (www.ncbi.nlm.nih.gov/genbank/). One caveat: The robustness of species identification based on DNA homology depends on ensuring the published sequence is associated with a correctly identified species.

(4) Immunological techniques are based on the detection of specific antibodies to proteins or other molecules produced by a pathogen species. These techniques, including the enzyme-linked immunosorbent assay (ELISA) and lateral flow device (LFD), showed higher diagnostic sensitivities than that of culture-based morphological identification, which can be influenced by environmental conditions (Lane et al. 2010). ELISA tests are generally inexpensive and relatively easy to perform, which makes them suitable for large-scale prescreening. On the contrary, LFD tests are more expensive and are not suitable for large-scale testing. Their strength is that they are rapid and robust, and can be used outside the laboratory (Lane et al. 2010). A general limitation of these techniques is that the antibodies used for ELISA and LFD rarely are species-specific and often cross-react with several *Pythium* species (Timmer et al. 1993).

Control or mitigation of extant *Phytophthora* infestations deserves its own review, but an excellent synthesis of approaches has been provided by Hayden et al. (2013), and we refer the reader to such a review.



The undersides of these petri dishes filled with *Phytophthora*-selective growth medium show *Phytophthora* colonies growing out of baits.

Phytophthora species possibly detected in restoration sites

As of the summer of 2017, at least 25 soilborne *Phytophthora* species have been recovered in restoration sites near natural ecosystems or in parks in the greater San Francisco Bay Area in California. Eight species are well known, eight are closely related and belong to Clade 6, and nine represent new putative hybrid species (see supporting table S1 online, http://ucanr.edu/u.cfm?id=215, for a partial list). All identifications were done both on cultures in vitro, and were based in part on morphology and in part on the homology of DNA sequences between published sequences and sequences of newly obtained isolates at the species-specific loci ITS and/or COX (Martin et al. 2012). Identification of novel *Phytophthora* species, their hosts or substrates and the California counties in which these species were found is still being completed, and, as a result, the information provided in table S1 should be taken as provisional and subject to change. Contributors of unpublished data are acknowledged in the acknowledgements section at the end of this review.

Please note that as this review is being written, more *Phytophthora* species are being discovered in California wildlands and parks; however, these species are not included here because they have not been shared yet by their identifiers. Also, note that the distribution information in this review is simply limited to the few areas that have already been surveyed. Hence, the actual distribution of the *Phytophthora* species included in this paper may be much larger than that reported here and may increase as more surveys are completed. Additionally, the taxonomy of these species is in flux, and thus their species designation may change in the future.

A provisional and partial list of soilborne species isolated in sites in Northern California as of the summer of 2017 includes, in alphabetical order, *P. bilorbang*, *P. cactorum*, *P. chlamydospora*, *P. cinnamomi*, *P. citricola*, *P. crassamura*, *P. cryptogea*, *P. erythroseptica*, *P. gonapodyides*, *P. inundata*, *P.* ‘*kelmania*’, *P. lacustris*, *P. megasperma*, *P. plurivora*, *P. quercetorum*, *P. riparia* and *P. tentaculata*. Nine hybrid species were also identified, but their precise diagnosis is yet to be completed, so we prefer to omit them. Table S1 provides a comparative analysis of the species listed in this review, for a range of important traits.

Phytophthora diseases are no longer limited to the ornamental plant production industry or to agriculture, but are also emerging as a complex issue in wildlands.

In conclusion, *Phytophthora* diseases are no longer limited to the ornamental plant production industry or to agriculture but are also emerging as a complex issue in native plant production and deployment. These diseases are emerging not only in association with inadvertent casual introductions, or due to the proximity of wildlands to agricultural settings, but also, unexpectedly, in association with infested plant production facilities providing stock for restoration projects and thus with restoration projects themselves. The problem is compounded by several issues, including (1) our inability to properly sample plant stock and the need for new sampling approaches (see Swiecki et al. 2018, page 217 in this issue), (2) the realization that *Phytophthora* species are in a continuum ranging from impossible to culture to easily culturable, (3) the fact that geographic distribution and the host ranges of *Phytophthora* species are not clearly known and are constantly changing, (4) the discovery of novel species at a faster pace than ever before and, finally, (5) reports that species forced to comingle in production facilities and in infested wildlands may generate new hybrid entities.

Early detection and understanding that there is a *Phytophthora* problem in Northern California remain key tools for mitigating and preventing further infestations. This will require that the scientific community continue raising awareness about this emerging problem and familiarizing stakeholders with details of some of the *Phytophthora* species that are increasingly being found in California wildlands. CA

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RESEARCH ARTICLE

Three new *Phytophthora* detection methods, including training dogs to sniff out the pathogen, prove reliable

A scent detection dog identified *Phytophthora* in media with a 100% accuracy; two other simple and cost-effective methods detected the pathogen with great confidence directly from plants.

by Tedmund J. Swiecki, Matt Quinn, Laura Sims, Elizabeth Bernhardt, Lauralea Oliver, Tina Popenuck and Matteo Garbelotto

Abstract

Multiple species of *Phytophthora* have been identified in production facilities of plants used in reforestation and restoration projects. There's a risk that infected plant stock will lead to *Phytophthora* species establishing and spreading in habitats that, having never experienced their presence, may be highly susceptible to infection. Eradication of these pathogens, once introduced into wildlands, is impossible. Thus, monitoring nursery stock is key, but sampling large production lots is still prohibitively complex and expensive. We tested three new sampling approaches that are practical for large production lots: baiting of small portions of symptomatic plant material pooled from multiple samples in addition to whole plant sampling; baiting of bench irrigation leachate; and training dogs to identify the pathogens. The first two methods detected *Phytophthora* with a high confidence level directly from batches of plants, but they are not designed to identify each infected plant specifically. Trained dogs identified individual batches of soil and water containing *Phytophthora* with a 100% accuracy and the research is continuing, to see if dogs can recognize the pathogen from individual infected plants and plant parts and discriminate its smell from other scents.

The introduction of microbial plant pathogens into natural ecosystems via contaminated stock has been observed multiple times (Geils et al. 2010; Grünwald et al. 2012; Jung and Blaschke 2004; Santini et al. 2013). Recently, it occurred in California, when *Phytophthora*-infected plant stock was used in restoration projects (Rooney-Latham et al. 2015).

The consequences are significant, particularly because many *Phytophthora* species are generalists and, as such, can easily "jump" across multiple hosts, potentially decimating those that are most susceptible (Garbelotto et al. 2018). The resulting plant mortality can erode the suitability of habitats for wildlife and other plants or for symbiotic organisms, resulting in cascading systemwide effects (Frankel et al. 2018). Infected stock may be distributed across a great area; the number of restoration projects in California is exceedingly large and a count is virtually impossible. For example, hundreds of restoration projects exist just in wetlands in the San Francisco Bay Area

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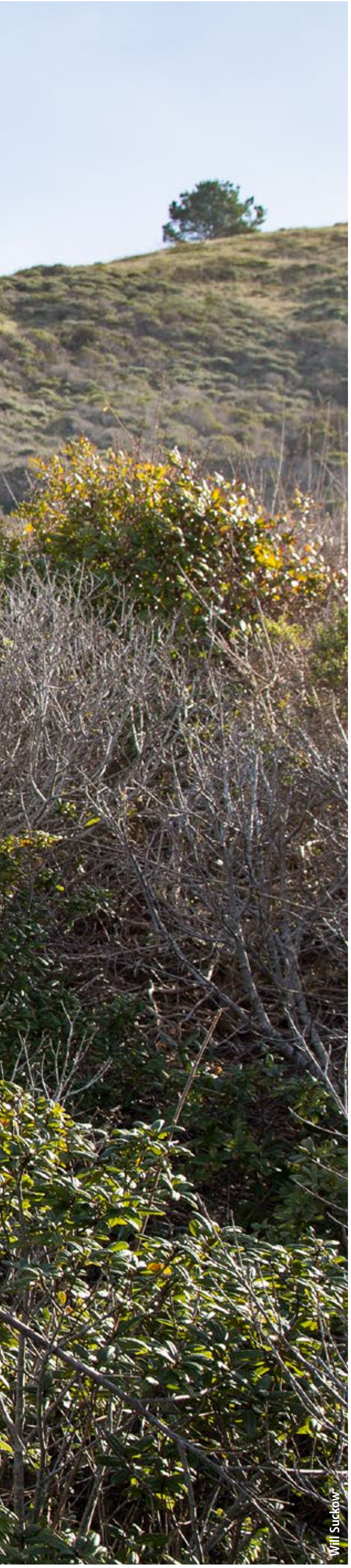
Co-author Laura Sims examines coffeeberry (*Frangula californica*) infected by *Phytophthora* introduced by nursery stock at a restoration area on Mori Point, Pacifica, in San Mateo County. The dead branches of coffeeberry in the foreground and background were killed by *Phytophthora*.

Will Suckow



(http://ca.audubon.org/conservation/restoration-projects). Likewise, we estimate there are over one hundred plant production facilities in California that provide plant stock for restoration efforts.

The problem of *Phytophthora* in plant production facilities has been compounded by several factors: asymptomatic infections (Osterbauer et al. 2004), the inability to correctly identify species due to a recent increase in the number of species (Kroon et al. 2012) and the use of chemicals that mask the infection without effectively eliminating the pathogens (Shishkoff 2014).

Molecular techniques have greatly enhanced the ability to correctly diagnose *Phytophthora* species (see Martin et al. 2012), and they have become cost effective for diagnosis at the species level on infected plants. The biggest hurdle is how to identify infected plants in large production lots, and how to adequately sample plant production facilities, many of which include large numbers of possible plant hosts.

Monitoring nursery stock to detect introduced pathogens is a key component of clean nursery production practices. To produce plants free of *Phytophthora* root rots to the maximum degree possible, a nursery needs to detect low levels of infection reliably. Any lapse in phytosanitary procedures must be identified and corrected quickly, so an infected plant can be quarantined to prevent disease spread in the nursery. And yet *Phytophthora*-infected plants may not show obvious symptoms in the canopy until root rot is severe, so visual inspection alone may not allow a nursery to catch an infestation at an early stage. To be practical, *Phytophthora* detection methods need to be relatively inexpensive and simple to carry out.

Our research studies in California have focused on different approaches to sampling for *Phytophthora*. The first study (Sims and Garbelotto) described and tested an assay approach in which portions of plants were collected and pooled to detect infection, and also included sampling from whole plants. The second study (Swiecki and Bernhardt) focused on a completely nondestructive detection method that tested irrigation water draining from plants. The third (Quinn, Oliver, Popenuck and Garbelotto) trained dogs to identify *Phytophthora* inoculum based on olfactory detection. For all three studies, this is the first published report on their outcomes, and, as such, its conclusions should be regarded as preliminary.

Detecting *Phytophthora* in root samples

In 2015, workers at the Presidio Native Plant Nursery (San Francisco, Calif.) noticed symptoms of severe and widespread root disease in a crop of blueblossom (*Ceanothus thyrsiflorus*), a common woody California native species. We capitalized on the availability of this infected blueblossom crop to perform this study. Our goal was to identify as many *Phytophthora* species as possible.

First, we evaluated the crop's actual infection level, commonly referred to as disease incidence value, caused by any combination of *Phytophthora* species. Then, we used the actual disease incidence values to calculate the minimum number of samples needed to detect the pathogen with a 95% confidence level using two sampling techniques (whole plant versus a composite of plant parts, see below). Finally, we created five sampling scenarios, using a combination of the two techniques, and estimated costs associated with each scenario.

Knowing the disease incidence of a crop and the detection rate of any given sampling technique are two essential pieces of information when attempting to design cost-effective sampling strategies. Disease incidence (DI) refers to the proportion of a lot that is infected and is a metric that will be positively correlated with the likelihood of discovering that any given production lot is infected. The detection rate (DR) is the proportion of infected plants that will test positive when measured by a specific methodology. In simple terms, the diagnostic effectiveness (DE) will be given by DE = DR/DI. A valuable assay necessarily needs to have a DE > 1.

Whole plant sampling is an intuitive and destructive sampling approach. Composite sampling combines small samples of roots and soil from multiple plants (25 in our case) into one sample, in a way not destructive to plants. Composite samples change the detection rate by improving it, and save money in lab processing fees by reducing the number of samples needed.

Calculating *Phytophthora* detection rate for a crop with a given disease incidence

There were 400 obviously symptomatic plants in a crop of 1,000 blueblossom plants. These were separated from the asymptomatic 600 and used to determine both the disease incidence and the detection rate as described below. Symptomatic plants were grouped by random selection into five blocks of 80 plants each, and *Phytophthora* was identified from each block following the standard techniques outlined below.

A total of 125 plants (25 plants selected randomly per block) were destructively sampled by baiting the entire plant. Baiting was performed as follows: Deionized water was added until it reached approximately 1 inch above the top of the sample; baits made of hard green pear fruit were submerged in the water over the soil and incubated for 5 to 7 days at a cool room temperature (18°C to 24°C), and moved to cold storage if temperatures exceeded this for several hours (Sims et al. 2015).

Each sample was baited separately, including control samples. Lesions on baits were plated on a *Phytophthora*-selective medium, kept at 18°C and identified using standard identification techniques at 3, 7 and 10 days after plating (Sims et al. 2015). Control baits were always negative. Cultures from positive baits were set aside for DNA extraction and storage. DNA was extracted using the Qiagen DNeasy Plant Mini Kit, PCR amplified using ITS (internal transcribed spacer) primers ITS4 (White et al. 1990) and DC6 (Cooke et al. 1999) and standard PCR settings (Sims et al. 2015). The amplified product was then sequenced and compared to published sequences in the GenBank database to determine species-level identification.

The overall disease incidence was calculated based on the baiting results from the 125 whole plant samples. The infection rate value was then used in power equations (Crawley 2007) to determine the smallest number of individual plant samples, n_1, that would be necessary to make the probability of missing the *Phytophthora* altogether less than 0.05 (5%), by solving:

$$0.05 = (1 - \text{infection rate})^{n_1} \quad (1)$$

Taking the logs,

$$\log(0.05) = n_1 \log(1 - \text{infection rate})$$

Therefore

$$n_1 = \log(0.05) / \log(1 - \text{infection rate}) = \text{minimum acceptable sample size}$$

To test the composite sampling technique, 25 plants from each block were selected at random and a small sample of roots and soil from each container was combined into a single composite sample. *Phytophthora* was identified from each composite sample following standard techniques (outlined above), and then the detection rate was determined. Finally, the detection rate was used, in the same way the disease incidence was used, to determine the smallest sample needed, n_2, to be 95% certain that the *Phytophthora* was captured. This result was achieved by solving:

$$0.05 = (1 - \text{detection rate})^{n_2} \quad (2)$$

Taking the logs,

$$\log(0.05) = n_2 \log(1 - \text{detection rate})$$

Therefore

$$n_2 = \log(0.05) / \log(1 - \text{detection rate}) = \text{minimum acceptable sample size}$$

Hybrid approaches and five sampling scenarios are presented; these were calculated using the sample density equation *dbinom*, using a saddle point algorithm for the greatest accuracy in the calculation of binomial probabilities (Loader 2000). All analyses were done using the R computing environment (R Development Core Team 2017).

Finally, rather than having a fixed disease incidence, we asked how the confidence level would change with varying infection levels. This was computed by solving equations 1 and 2, above, for the probability of missing *Phytophthora*.

Validation of the assays

Table 1 summarizes the calculated disease incidence and sample size necessary to detect the infection with a 95% confidence level. Whole plant sampling across all five blocks determined that the crop's infection rate was 28%. A minimum of 10 whole plant samples or four composite samples (if a composite includes portions from 25 plants) are necessary to detect the infection at this confidence level and infection rate.

Scenarios with a combination of whole plant and composite samples were also calculated to achieve the same 95% confidence level of detection probability. The same statistical confidence is achieved using different sample sizes because the detection rate per sample varies on a sliding scale in different scenarios (sliding scale average detection rate per sample, or SSDR; see table 1). Finally, costs were computed (table 1) for each sampling scenario, to provide an additional parameter for selecting the most appropriate one.

When disease incidence changes, so does the statistical confidence of these sampling scenarios. Of course, if disease incidence is higher than 28%, then confidence

TABLE 1. Five scenarios for detecting *Phytophthora* using whole plant and composite sampling to achieve a 95% confidence level, with a disease incidence of 28%

Approach	Confidence level of detection rate per crop	Sampling time*	Lab costs†	Sliding scale detection rate per sample (SSDR)	Samples per crop	Constant disease incidence	Samples
A	95%	20 min	$500	28%	10	28%	10 whole plants
B	95%	39 min	$400	32%	8	28%	1 composite sample + 7 whole plants
C	95%	60 min	$350	37%	7	28%	2 composite samples + 5 whole plants
D	95%	79 min	$250	52%	5	28%	3 composite samples + 2 whole plants
E	95%	100 min	$200	60%	4	28%	4 composite samples

* Estimates 2 min to collect a single plant for sampling and 25 min to collect a composite sample.
† Assumes $50 per sample for lab processing.

of the sampling approaches increases, but if it is 20%, for example, confidence becomes too low (80%) and the scale of the sampling would need to be increased to achieve the necessary 95% confidence level (see table 2).

Collecting the samples

In a production facility, collecting the samples consists of the following steps. First, determine whether disease symptoms are aggregated in groups of symptomatic plants or are scattered randomly. If symptoms are aggregated in clusters, count the number and determine the location of each cluster, to ensure all clusters are sampled equally. Mark plants with any visible symptoms, collect these plants and place them together in a single area of the production facility. Then, once gathered, generate a randomized list (with plant tag numbers) to select plants for sampling. Tag each plant with a unique tag number, sampling evenly across the strata if aggregated. Designate the first plants on your list for whole plant sampling, and the second set for composite sampling, again ensuring sampling across strata if necessary.

TABLE 2. Probability of at least one *Phytophthora* detection from a production lot with varying disease incidence

Disease incidence	Probability of at least 1 detection
40%	> 98%
35%	> 98%
30%	> 95%
25%	> 95%
20%	> 80%
15%	> 75%
10%	> 65%
5%	> 40%

All plants used for samples should be fully developed so that roots reach the outer portion of containers. For each sample, place roots and soil in a single leak-proof 1-gallon bag. Label each bag with a unique identifier that tracks it back to the original plants and notes associated with the sample and crop. After preparing each sample, add deionized water to 1 inch above the sample line and add washed unripe pear bait.

Plants selected for whole plant sampling should be grouped together. Remove each plant from its container to expose the root system. Include portions of the primary root ball and of the soil from all areas within the container, until you have reached a total sample of soil and roots of approximately 2 liters. Be sure to include samples of degraded roots from all areas within the container. When done, place the whole sample in a leak-proof 1-gallon bag.

Plants selected for composite sampling also should be grouped together. Use a Scoopula to remove 2 tablespoons of roots and soil along a lateral gradient from the upper to lower portion of the container at the container edge of each selected plant, from two opposite sides. In between each plant, wipe your tool with 70% alcohol. Place sample roots and soil in a bag and mix, and then the sample is ready for baiting.

Detecting *Phytophthora* in leachate

Phytophthora infections can be identified by detecting swimming zoospores released from infected roots into bench leachate. This identification method takes advantage of two well-established facts. First, irrigation runoff from *Phytophthora*-infected plants carries zoospores that are detectable by baiting (MacDonald et al. 1994). Second, zoospores tend to swim upward in a water column (Erwin and Ribeiro 1996), a phenomenon known as negative geotaxis (movement in the opposite direction of gravity), which helps concentrate zoospores from large volumes of leachate.

Phytophthora spreads very efficiently in nurseries, so it is important that an infection is detected quickly; once the pathogen is detected, the entire block of plants must be quarantined or disposed of. The bench leachate test is a quick test; a block of many plants can be tested at once, rather than requiring multiple individual plant tests. The test also potentially detects infection anywhere within the root systems of plants in a block, rather than from a targeted sample of root tissue from a selected set of symptomatic plants. Plants can be tested in place on a nursery bench or moved to a cart or another bench for testing.

Conditions for a sensitive test

The test depends on *Phytophthora* sporangia being present in the plant root systems or potting media and releasing zoospores during the test period. To maximize test sensitivity, conditions before the test need to be favorable for sporangia production and for zoospore release and motility.

Prior to testing, plants should be irrigated regularly, because viable sporangia may not be present if plants have been dry for an extended period. This precondition is typically met in most nurseries. Testing should also be conducted when average soil temperatures have been in the range of 65°F to 75°F (18°C to 24°C) for at least 3 days, preferably a week or more. These temperatures are favorable for growth and sporangium production in a wide range of *Phytophthora* species (Erwin and Ribeiro 1996).

It is possible to detect some *Phytophthora* species at temperatures outside of this temperature range, but our studies (data not shown) indicate that some species are less likely to be detected if soil temperatures are well above or below this range. For the same reason, the temperature of irrigation water applied during the test should be between 50°F (10°C) and 77°F (25°C). Unless plants are grown in controlled environments such as greenhouses, testing should be scheduled to avoid overly hot or cold conditions.

Conducting the leachate test

A collection system is placed beneath a mesh bench containing the plants to be tested. During irrigation, leachate from the bottom of plant containers is directed into a zoospore collection vessel (ZCV). As the ZCV

Zoospore collection vessel used in the leachate detection study. The drain is 2.75 inches (7 centimeters) above the bottom; the water outflow (upper pipe elbow outside of vessel) is situated to maintain the water level about 2.5 inches (6 centimeters) below the rim. Vessel depth is 12 inches (30 centimeters). A pool thermometer (shown) or similar can be used to monitor water temperatures during the test.

fills, water drains from the lower-middle portion of it rather than overflowing at the top, which would result in loss of upward-swimming zoospores. The vessel also captures debris, which floats or settles to the bottom of the vessel and may contain sporangia.

Green pears are used as detection bait. Green pears are readily available year-round and can be infected by a wide variety of *Phytophthora* species. If unwounded, they are also highly selective for *Phytophthora* species, which commonly induce distinctive lesions. Most pears float at the water surface, where they attract motile *Phytophthora* zoospores. For the occasional nonfloating pear, a pear floatation device can be made by using a rubber band to attach a small piece of closed-cell foam.

At the start of the test, a green pear is placed into the ZCV, which is situated to receive the irrigation leachate channeled by the collection system. Plants are individually irrigated six times at 15-minute intervals, using low pressure to avoid splash. Applied irrigation should not overflow the container rim but should be sufficient to cause water to leach from the bottom of each container. Approximately equal amounts of water should be applied to each container. For #1 containers, the amount applied at each irrigation should be about 22 fluid ounces (650 milliliters); larger containers will take more and smaller ones less water at each irrigation. The irrigation regime is based on experiments showing that few if any zoospores are detected in leachate from the first two irrigations but are readily detected in leachate from irrigations 3 through 6 (table 3).

Fifteen minutes after the sixth irrigation (about 90 minutes after the first irrigation), the pear is transferred to a heavy-duty 1-gallon zip-closure plastic bag supported in a container. Water in the ZCV is drained from the center of the water column until 2.9 quarts

Leachate collection systems and zoospore collection vessels under arrays of 42, *top*, and 21, *bottom*, #1 container plants. Each array contains one known *Phytophthora*-infected plant. Remaining pots are filled with pasteurized potting media (seeded with turfgrass, *top*).

TABLE 3. Detection of *Phytophthora cactorum* in leachate from 15 *Ceanothus thyrsiflorus* in #5 containers that included one infected plant

		Test 1		Test 2	
Irrigation number	Time from start of test	Baiting result	Days to first symptoms	Baiting result	Days to first symptoms
1 + 2	0 to 30 min	Negative	—	*P. cactorum* (1 lesion)	6 days
3 + 4	32 to 65 min	*P. cactorum*	5 days	*P. cactorum*	3 days
5 + 6	65 to 98 min	*P. cactorum*	7 days	*P. cactorum*	3 days

(or about 2.7 liters) remain; the amount shown is precise because this appears to be the maximum amount of water that will fit without spillage in the 1-gallon bag used for incubation. This remaining water, which typically includes container mix and other particles that have settled to the bottom, is then transferred into the zip-closure plastic bag with the bait. The pear remains floating in this water for 3 more days at moderate temperatures (65°F to 75°F, 18°C to 24°C). At that point, pears are removed from the water and placed onto clean paper towels for up to an additional 5 days of observation.

Our studies (data not shown) indicate that infection of the pear bait can occur in the first 90 minutes of the test (while the pear is in the ZCV) and in the following 3-day baiting period, so both parts of the test can contribute to a *Phytophthora* detection. Symptoms may develop on the pear bait in as little as 2 days after the test or may take as long as 5 days after the pear has been removed from the water (8 days after leaching). Culturing pieces from the pear lesions can be used to confirm *Phytophthora* detection and obtain a species identification. A detailed description of the protocol and equipment used is available at http://phytosphere.com/BMPsnursery/test3_4bench.htm.

Sensitivity of leachate test

We conducted a series of experiments to assess the sensitivity of this protocol. Into an array of containers with noninfected plants or containers with only pasteurized potting media, we placed one or two *Phytophthora*-infected plants. *Phytophthora* was detected when infected plants made up no more than about 6% of the array. *Phytophthora cactorum* was consistently (eight times in eight tests) detected from an array of 15 *Ceanothus thyrsiflorus* in #5 (3.8-gallon, 14.5-liter) containers with one infected plant. *P. niederhauserii* was detected in three of three tests from an array of 42 #1 (0.75-gallon, 2.8-liter) containers that included a single infected *Juniperus sabina* 'Tamariscifolia'; the total irrigation volume for that test array was about 43 gallons (164 liters).

In other tests conducted at soil temperatures of 62°F (17°C) or less, which is below the recommended minimum, detection of several different *Phytophthora* species was inconsistent. *Phytophthora* was detected in five of nine tests in arrays with 5% infected plants and in one of four tests in arrays with 2% infected plants. Most of the *Phytophthora* source plants in these studies were recently transplanted and were smaller than typical for #1 container stock. Hence, inoculum would be more diluted, probably by a factor of three or more, beyond what was expected based on the infected plant percentage.

Results from these and other studies suggest that the minimum threshold for detection can vary based on the species of *Phytophthora* present, temperatures of irrigation water and soil, and condition of the *Phytophthora* source plants. Additional studies are under way to assess how these and other factors influence detection efficiency and whether the protocol can be modified to minimize these influences.

To date, we have used the test protocol in multiple nurseries under various temperature conditions and have detected nine *Phytophthora* species in nursery stock from a range of plant species and container sizes. Results from these tests have enabled land managers to identify and prevent the planting of *Phytophthora*-infected material into native habitats. Several habitat restoration nurseries have implemented this protocol for testing stock they are producing for habitat restoration plantings.

Although we have detected *Phytophthora* in plant batches of up to 200 small containers (Deepot D40, 0.17 gallon, 0.66 liter), we suggest limiting the number of containers in a test batch to about 40 until more data from controlled sensitivity tests is available. Test sensitivity can be maximized by selecting the most symptomatic plants in a batch, rather than random

Pear baits during incubation in the leachate study, 3 days from test, *top*, and 1 day after removal from leachate, *bottom*. Pear at left in top image shows brown lesions caused by *Phytophthora cactorum* infections; pear at right has no *Phytophthora* symptoms. Bottom image shows a range of *Phytophthora* symptoms in pears, from a single spot (upper left) to extensive infections.

sampling. Plants should be tested when they are well established within a given container size, before rather than after they are moved into larger containers. This test does not produce false positives, but false negatives are possible. Repeated testing can provide greater confidence in negative results.

Detecting *Phytophthora* by smell

The UC Berkeley Forest Pathology and Mycology Lab teamed with H. T. Harvey & Associates to determine if it is possible to train ecological scent detection dogs to survey for the presence of *Phytophthora*. Although the research is scant, there are a few examples of dogs being successfully employed to detect plant pathogens (see Eckhardt and Steury 2012; Woollett et al. 2012). If dogs could detect *Phytophthora*, it would allow for more immediate confirmation of the pathogen than is available using current detection methods.

The team developed a *Phytophthora* detection dog pilot study, which includes a two-phase training approach, starting with a single dog (starting with one animal is the standard practice in the industry). The first phase of the scent recognition training focuses on teaching the dog to recognize *Phytophthora* odor in a range of media. The dog-handler team is introduced to two aqueous mixtures, potting soil and locally collected forest soil. We test the ability of the dog to detect four species of *Phytophthora* in those four media. Next, we test whether the dog can detect the same four species in an infected plant or plant parts (i.e., leaves and roots).

If that is successful, we commence the second phase, which is the scent discrimination phase. That involves conducting experimental trials in which targets and nontargets are manipulated to test the dog-handler team's ability to discriminate the scent of *Phytophthora* species from co-occurring and distracting scents.

Preparing the *Phytophthora* targets

All training and trials were conducted in the *Phytophthora* quarantine lab at the Forest Pathology and Mycology Lab in 2017. *Phytophthora* species were cultured in pea broth media designed to facilitate sporulation and were handled and stored by qualified lab staff members using *Phytophthora* quarantine procedures. All samples were handled using latex or sterile nitrile gloves and placed into a secured container with a ventilated lid. Two different types of containers were used during this study: PVC tubes placed over a ventilated container and glass mason jars with wire mesh lids. They were designed to allow the target's scent to be released while preventing the dog from touching the sample.

Four *Phytophthora* species were used for this study: two airborne species, *P. nemorosa* and *P. ramorum*, and two soilborne species, *P. cactorum* and *P. cinnamomi*. Each species was grown in standard pea broth (Erwin and Ribeiro 1996), by placing three disks, $^{15}/_{64}$ inches (6 millimeters) in diameter, in 12-well cell culture plates containing 5 milliliters of pea broth, and placing the plates in an 18°C incubator for 5 days. This inoculum preparation protocol resulted in the production of mycelium, sporangia and chlamydospores for all four species, and possibly in the production of oospores for the homothallic (i.e., self-fertile) *P. cactorum* and *P. nemorosa*. For each trial, different media were amended with three 30-cubic-millimeter samples of the inoculum of each species.

Training the dog to the scent

Initial training was conducted using the inoculum of each target *Phytophthora* species simply absorbed onto filter paper, to offer the dog the purest scent of *Phytophthora* possible. The dog was then exposed to *Phytophthora* inoculum placed in potting mix commonly used in commercial nurseries, in forest soil collected under California coast live oak (*Quercus agrifolia*) in Lafayette, California, in a mixture of soil and water and, finally, in a combination of the original pea broth used for the growth of the inoculum and the soil-water mixture.

The two types of containers used to hold *Phytophthora* during the training of the scent detection dog.

Container drills were used to teach the dog to associate the target odor with a play or food reward. The dog was led along a row of eight identical ventilated plastic containers. Four of the containers held *Phytophthora* species in one of the four media, and the others were control containers (identical containers that held the same medium but without the *Phytophthora* inoculum). When the dog sniffed the container with the target odor, she was rewarded immediately.

This exercise was repeated until the dog displayed anticipatory behavior when she smelled the target odor. Anticipatory, or "alert," behavior varies among dogs but often includes a sudden change in direction of movement or a change in posture, combined with focused attention toward the handler in anticipation of the reward. This behavior demonstrates to the handler that the dog associates the target odor with the reward, after which the dog is ready to be tested on her ability to consistently recognize the target scent.

After the dog displayed recognition of the target odor, a scent recognition test was performed in which the dog-handler team was required to successfully indicate one target container randomly placed in a linear arrangement with seven control containers. The handler was unaware of the placement of the target container. The dog-handler team was required to successfully complete 10 consecutive target detection trials to move on to the next species and medium.

Validation of the assays

The dog-handler team began training in the lab Feb. 2 and performed its first tests Feb. 16. Training and testing continued until March 9. A total of 16 tests were performed (four *Phytophthora* species in four media) (table 4). The team passed each test on its first attempt, achieving a positive alert only to the target container in each of 10 trials.

Results from the study so far suggest that ecological scent detection dogs may offer an innovative and reliable method to survey for *Phytophthora* in a variety of settings. Target recognition remained strong even when the shape and size of the containers were manipulated and the quantities of the pathogen varied. The dog excelled at communicating the locations of the pathogens to the handler, demonstrating a 100% detection rate.

Results to date engender confidence that detection dogs may offer an efficient and effective alternative or complementary technique to detect *Phytophthora*. Current detection techniques require various laboratory tests to confirm presence and identity, and ecological scent detection dogs could possibly be used in place of some lab tests. Dogs could offer a rapid way to reliably detect the pathogen in a variety of controlled environments, such as nurseries; to prescreen plants before they are installed at habitat restoration sites; and possibly to identify infected naturally occurring plants and soil in the field.

The next part of the scent recognition training phase will be to transition the dog to recognize *Phytophthora*-infected plants and plant parts. Infected rhododendron leaves and live plants with infected roots will be presented to the dog in the lab to assess her ability to detect *Phytophthora* in these living materials. Undoubtedly these tests will be more challenging due to a broader range of confounding factors.

Following completion of the scent recognition phase, the objective is to quickly progress to phase 2, the scent discrimination training. We will test the dog's ability to discriminate harmful *Phytophthora* species from co-occurring and distracting scents, including common related water molds, such as *Pythium* species.

TABLE 4. Results from scent detection dog study

Species	Substrate	Date completed	Success in classifying targets
P. nemorosa	Potting soil	Feb 24	100%
	Local soil	Feb 24	100%
	Soil-water solution	Feb 17	100%
	Soil-water/pea broth	Mar 9	100%
P. ramorum	Potting soil	Feb 23	100%
	Local soil	Feb 23	100%
	Soil-water solution	Feb 16	100%
	Soil-water/pea broth	Mar 9	100%
P. cactorum	Potting soil	Feb 16	100%
	Local soil	Feb 16	100%
	Soil-water solution	Feb 16	100%
	Soil-water/pea broth	Mar 9	100%
P. cinnamomi	Potting soil	Mar 3	100%
	Local soil	Mar 3	100%
	Soil-water solution	Feb 23	100%
	Soil-water/pea broth	Mar 9	100%

The dog excelled at communicating the locations of the pathogens to the handler, demonstrating a 100% detection rate.

Imprinting the odor of *Phytophthora* using a container drill.

Detection dog displaying anticipatory, or alert, behavior at the one container that contained *Phytophthora*.

Future directions

The studies presented here provide proof of concept on the potential of three distinct approaches to detect *Phytophthora* in production facilities with minimal destructive sampling of nursery stock. All three approaches are suitable for large production facilities specializing in the production of plant stock for restoration because they help to minimize sampling costs and plant damage while achieving measurable and often high detection levels.

The composite sampling approach validated in this study capitalizes on the presence of symptomatic plants to detect *Phytophthora* (Hayden et al. 2004). In some cases, asymptomatic plants may also be infected in nurseries (Bienapfl and Balci 2014; Parke et al. 2014). Other methods are available for randomly sampling plant lots with no symptoms (Bienapfl and Balci 2014), but they were not within the scope of our study.

In the case of the leachate approach, priorities for future research include assessing detection sensitivity in small container sizes commonly used in restoration plantings. Plants in these small containers are tightly packed in racks or trays and often produced in large quantities, so a high detection efficiency is desirable.

For dog-based detection, it will be necessary to successfully complete the scent recognition phase of the training and then progress to field tests under a range of conditions. Field tests will include plants whose infection status can be verified by other methods so that the frequency of false negative and false positive identifications can be determined.

Notwithstanding the need for further research, the approaches described appear to be innovative and powerful, with clear practical applications. Further work is needed to refine the approaches and determine the range of conditions under which they can be applied. Whether they are truly applicable as here described, or they need adjustment, can be determined only when sampling is performed at a larger scale and includes greater sample, facility and pathogen variability. CA

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Dependence on policy revenue poses risks for investments in dairy digesters

California dairy farms face policy uncertainties over investments in anaerobic manure digestion to produce methane for renewable, low-carbon vehicle fuel.

by Hyunok Lee and Daniel A. Sumner

Abstract

Manure-sourced methane emissions from livestock operations in California will soon be subject to new regulation, as required by Senate Bill 1383, which was signed into law in 2016. Regulations, beginning in 2024, will require reductions in methane emissions from livestock manure, with a 40% reduction target by 2030. The California dairy industry accounts for most of the manure-sourced methane emissions in the state and, in order to reduce these emissions, government experts and authorities have encouraged expansion of anaerobic digestion of dairy waste — especially to produce transportation fuel. Renewable natural gas for vehicle fuel, produced from manure at digesters, is eligible for substantial federal and California environmental credits, which are now projected to contribute the bulk of the revenue for qualifying digesters. This article shows that investments in digesters, because they depend heavily on revenue created by government policy, rather than on market-based sales of natural gas, are highly vulnerable to the risk of policy change or even minor technical adjustments in environmental regulations. Without secure projections of revenue that will cover costs, regulations may cause increases in the shift of milk production out of California.

Milk is central to California's agricultural sector and the state is central to the U.S. dairy industry. Milk is the largest California farm commodity by sales value and California is the nation's top dairy state, with substantial overseas exports of milk products. However, the dairy industry faces many economic and policy challenges, none of which is more vital than how to deal with myriad environmental concerns and related regulations. Water and air quality issues have drawn the attention of state authorities such as the California Department of Food and Agriculture (CDFA), the California Air Resources Board (CARB) and the California State Water Resources Control Board. CDFA and CARB, among other agencies, are in the midst of an aggressive attempt to reduce greenhouse-gas emissions from agricultural production and processing. Their regulatory proposals are designed to meet legislative mandates while minimizing negative economic impacts.

Under a recently passed law that is now in the implementation process, California livestock farms will

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Cows idle at a confinement dairy in Fresno County. For dairies with confinement housing and herd sizes often in excess of 1,000 cows, it can be challenging to handle manure in environmentally sound and economically sustainable ways. The most common approach, known as the flush-to-lagoon system, produces large amounts of methane, a powerful greenhouse gas. A California law passed in 2016 mandates steep reductions in methane emissions associated with dairy manure.

Elena Zhukova

soon be subject to state regulations on greenhouse-gas emissions. As mandated by Senate Bill (SB) 1383 (Lara 2016), which was signed into law on September 19, 2016, methane emissions associated with manure produced at California livestock operations will be subject to detailed regulations, which will be phased in beginning in 2024.

The California livestock industry — particularly the dairy industry — is a significant contributor to the state's methane emissions. Methane is produced and emitted when ruminants digest by enteric fermentation and when livestock manure decomposes under anaerobic conditions. Livestock manure management has been subject to federal, state and local environmental regulations for many years, but regulation to mitigate methane emissions is new. SB 1383 calls for mandatory regulation of manure-sourced methane emissions by 2024 in order to reach a 40% reduction of greenhouse-gas emissions by 2030. Implementation of such regulations is conditional on economic feasibility among other conditions (Lara 2016). Enteric fermentation, although a larger source of methane emissions in California, is not yet subject to regulation.

Prior to the passage of SB 1383, in response to a legislative request, CARB initiated a study to develop comprehensive strategies for controlling short-lived climate pollutants, one of which is methane. Among several possible technologies for controlling manure-sourced emissions, CARB identified as most favorable a system of centralized digesters that would produce pipeline-injectable biomethane or renewable natural gas. CARB determined that such a system would be more favorable than the alternatives — not only financially but also in terms of achieving the large-scale methane reductions that are required by SB 1383 (CARB 2017). The system would be comprised of 55 digesters that would each collect manure from a cluster of nearby farms, process that manure and produce marketable methane. Under CARB's scenario, such a system would operate in the heart of the San Joaquin Valley's intensive dairy region — allowing economies of scale while still limiting the cost of manure transport.

Alternatives considered by CARB included a system in which a digester on each farm would produce biogas that would be piped at low pressure to a locally centralized facility — which in turn would process the biogas, producing renewable natural gas for vehicle fuel. That system would entail some advantages, such as enabling more convenient use of nitrogen from effluent on farmland. But CARB assessed the system's financial feasibility as lower than that offered by its preferred system of moving manure to locally centralized digesters.

This article achieves five specific objectives: (1) documenting the current methane emissions of the California dairy industry, (2) describing the economic attributes of several digester technologies, (3) summarizing, in a useful framework, economic data regarding the system of digesters that CARB has identified as feasible for the San Joaquin Valley, (4) explaining and examining some key policy and economic assumptions, related to government policies on biofuel credits, that are built into CARB's economic evaluation of digesters and (5) explaining how those assumptions influence the ways in which investments in digesters may affect the economics of the California dairy industry. The overall goal of this study is to analyze and explain the economic circumstances that California dairy farms will encounter as they begin to comply with the impending regulations.

Methane and livestock

Figure 1 shows California methane emissions by source in 2013, which under SB 1383 is the benchmark year for livestock methane emission regulations, and which is used by CARB as well. Livestock accounts for 54% of California's methane emissions, primarily because the dairy industry is so large in the state.

Livestock generates methane emissions by two means: enteric fermentation and manure decomposition. Enteric fermentation creates methane in the digestive systems of ruminants such as cattle, sheep and goats. This methane is later emitted, primarily when the animals exhale or belch (Moraes et al. 2014). Methane is also generated during anaerobic (without

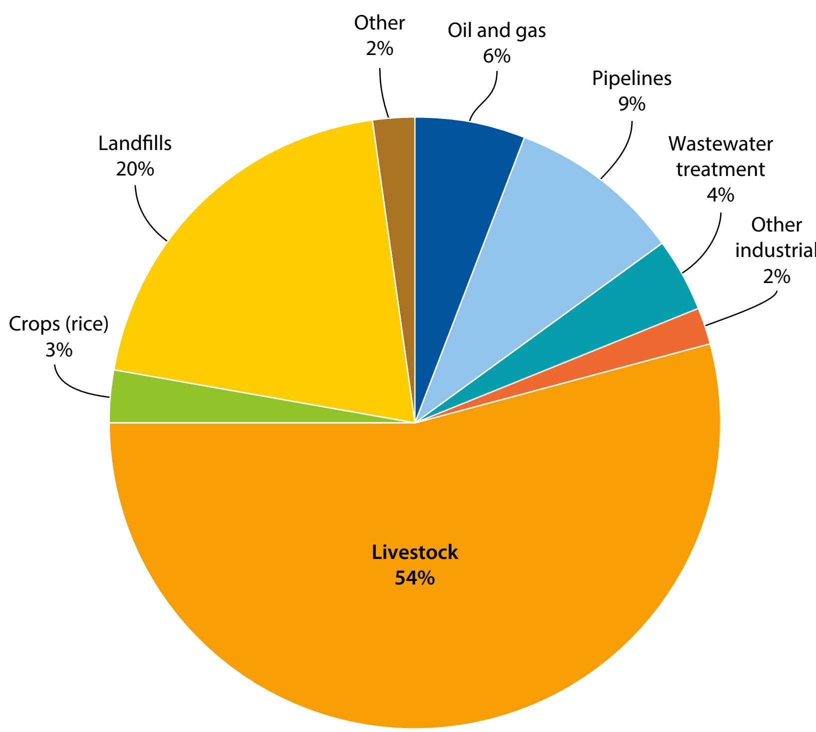

FIG. 1. California methane emissions by source in 2013 (total = 118 MMT CO_2e) Source: CARB (www.arb.ca.gov/cc/inventory/slcp/slcp.htm). Emission numbers for short-lived methane are based on the Global Warming Potential definition from the 2007 Intergovernmental Panel on Climate Change Fourth Assessment Report (20-year Global Warming Potential).

air) decomposition of manure. Anaerobic decomposition of manure is common at confined animal facilities such as the many large dairies in California that process manure in lagoons.

Figure 2 breaks down by source the methane emissions associated with livestock in California. Manure handling contributes 47% of livestock methane emissions, with the dairy industry alone contributing 45%. Enteric fermentation contributes the remaining 53%, with the dairy industry accounting for 37%. The dairy industry accounts for 82% of overall livestock methane emissions in the state, with the beef industry accounting for almost all of the remainder.

Technologies for controlling livestock methane

Methane emissions from enteric fermentation can be reduced by altering ruminants' diets. However, the biological relationships among emissions, health and nutrition in the context of alternative diets are complex (Liu et al. 2017; Moraes et al. 2014; Veneman et al. 2015). Research in this area is under way, but the potential to effectively and feasibly reduce methane emissions associated with enteric fermentation is currently limited. This is one reason that SB 1383 does not mandate reductions in emissions associated with enteric fermentation.

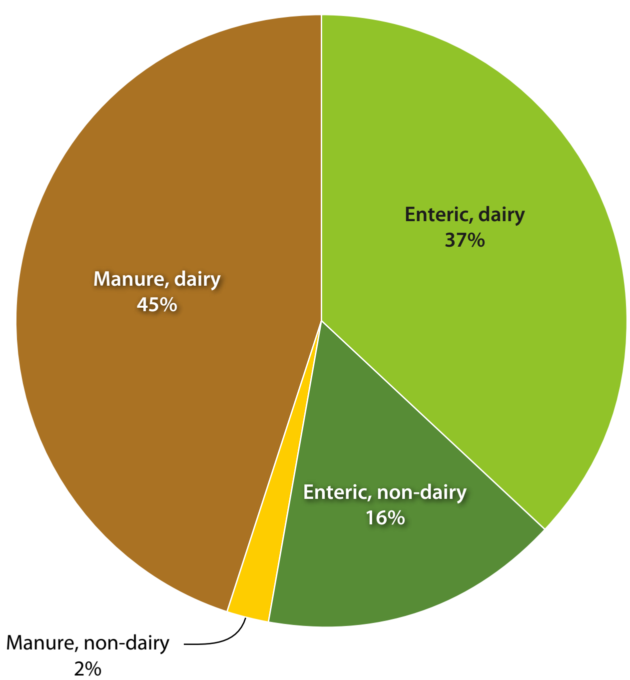

FIG. 2. California livestock methane emissions (total = 64 MMT CO_2e). Source: CARB (www.arb.ca.gov/cc/inventory/slcp/slcp.htm). Emission numbers for short-lived methane are based on the Global Warming Potential definition from the 2007 Intergovernmental Panel on Climate Change Fourth Assessment Report (20-year Global Warming Potential).

Manure-based emissions can be reduced through changes in manure management. Analysis of approaches to manure management has been under way for decades, and many technologies have been evaluated under many conditions. Recent studies have reviewed and evaluated several methods by which California dairy manure management could be modified to reduce methane emissions (CARB 2017; Kaffka et al. 2016). Methods evaluated include (1) increasing the prevalence of pasture-based dairy farming, (2) scraping and drying manure and (3) using anaerobic digestion to further process manure.

Dairy cows are often raised on pasture in places such as New Zealand and parts of Australia, and in the small dairy industry remaining in the North Coast region of California. Manure dries when left on pastures and is incorporated into the environment with little methane emission. That is also why manure-related methane emissions are low for most beef cattle in California, which are raised on pasture.

Despite its use elsewhere, pasture-based milk production is not well suited for large-scale adoption in California; it lacks economic feasibility except in specialized situations. Unlike in New Zealand, say, where the dairy system has adapted to the wet climate, California rainfall patterns create insufficient areas of high-quality pasture — especially in the major dairy region of the state, where irrigation water is limited and expensive. In addition, milk per cow is typically much lower when pasture is used for forage. In California, pasture-based dairy forage (supplemented with hay and silage) has been economically feasible only for relatively small dairies located in the North Coast region. Over time, this region's share of California milk production has declined. Pasture-based dairies in California now typically sell organic milk, or sell milk for use in specialty products destined for high-priced niche markets. Even in California locations well suited to pasture-based dairy farming, production costs are high. At California's pasture-based dairies, cost per unit of milk output is about 70% higher than the cost at confinement dairies in the San Joaquin Valley, where more than 90% of California milk is produced (CDFA 2017).

Dairies with confinement housing and herd sizes typically exceeding 1,000 cows face challenges in handling manure in environmentally sound, economically sustainable ways. One approach is to scrape manure daily from pens and barns, using vacuum trucks or mechanical scrapers (Kaffka et al. 2016), and then to dry the manure to a solid form. This approach produces lower methane emissions than does the commonly used flush-to-lagoon system, but the manure-drying process has to comply with regulations, such as building codes and local water quality rules, that prohibit leaching. The scrape-and-dry method can be costly for large commercial dairies in California (CARB 2017; Kaffka et al. 2016).

Unlike the pasture-based or the scrape-and-dry manure handling systems, anaerobic digestion allows

Methane captured from manure at a covered lagoon dairy digester in Sacramento County is later processed for electricity generation. In the San Joaquin Valley, the need to comply with federal air-quality regulations means that using biogas for on-farm electricity generation is an expensive way to reduce dairy methane emissions.

production of methane — but it recovers, or captures, the gas that would have otherwise been emitted. The captured biogas can be used to produce renewable electricity or processed to produce renewable pipeline-quality natural gas (or renewable compressed natural gas). This energy can then be used in the operation on site or marketed to customers elsewhere.

Anaerobic digester technology typically requires large investments of financial and human capital. In addition, the efficient development and operation of a digester often requires substantial time and managerial expertise in an area other than dairy farming. (A detailed review of studies that discuss economies of scale and related economic issues involved in digester technology is provided by Lee and Sumner [2014]). Nonetheless, because of its potential to produce renewable (or low-carbon) energy, the digester approach has garnered considerable attention among environmentalists, policymakers, technology advocates and potential investors (Lee and Sumner 2014).

Anaerobic digester technology

Biogas is a natural product of any anaerobic digestion of organic material. With methane as its primary component, biogas can be processed for use in several applications. It can be combusted to produce electricity, heat or both. It can be cleaned and upgraded into pipeline-quality biomethane (also known as renewable natural gas, which qualifies as a cellulosic biofuel under a federal program, the Renewable Fuel Standard [EPA 2017b]). For use in vehicles, renewable natural gas is typically compressed and used in the form of renewable compressed natural gas.

For many years, electricity has been the most common energy output produced from biogas. However, using biogas to generate electricity has been more challenging in California than in other parts of the country. Combustion of biogas during electricity generation emits nitrogen oxides (NOx) — substances regulated in locations, such as the San Joaquin Valley, that are ozone nonattainment areas under rules established by the federal Clean Air Act (EPA 2017a). Complying with NOx regulations generally requires using either costly emission control technologies or expensive electricity generation technologies such as microturbines. The need to comply with federal regulations has meant that on-farm electricity generation using biogas has been an expensive strategy for reducing dairy methane emissions in the San Joaquin Valley.

A recent California policy change enhanced the potential payoff for dairy digesters that produce renewable natural gas.

An alternative use for biogas is as pipeline-injectable renewable natural gas. The process of cleaning and upgrading biogas and distributing it through a pipeline, however, is quite capital intensive. With significant economies of scale, operations on a large scale are needed to reduce costs per unit. The number of cows required to reach reasonably low per-unit costs is usually greater than the number of cows at even the large California milk cow facilities. With the concentration of large dairies in the San Joaquin Valley, however, neighboring dairies can form a cluster that supplies manure as a raw material to a locally centralized digester, where biogas can be generated and processed. With a reasonable number of clusters operating in the San Joaquin Valley, locally centralized digesters may have the potential to curtail methane on a large scale — by as much as the 40% called for under SB 1383.

TABLE 1. Number of digesters in California: newly constructed, shut down and operational, 2006–2018 (as of April 2018)

Year	New	Shut down	Existing
2006	2	0	12
2007	1	1	12
2008	4	1	15
2009	2	6	11
2010	0	1	10
2011	0	0	10
2012	0	1	9
2013	5	0	14
2014	2	0	16
2015	1	3	14
2016	2	1	15
2017	2	0	17
2018	3	0	20

Source: EPA (2018a).

History of digester investment

Digester technology has been available and in use for decades, but it has not been widely adopted in California. All dairy digesters that have operated in California have received substantial support from federal and state government in the form of grants, favorable loan arrangements and other incentives. For projects examined in case studies, grants have averaged more than 40% of capital cost (Lee and Sumner 2014).

Table 1, covering the period from the beginning of 2006 through April of 2018, shows how many California digesters were newly opened or shut down in each year — as well as the total number of digesters operating each year. Over this 12-year period, new digesters were regularly built, supported by infusions of public funds. Then, after a few years, many were taken offline. All current digesters are dairy-based operations, with the number of cows ranging from 400 for a Marin County digester to 15,500 for a Kern County digester. Some facilities practice codigestion, an approach in which, along with dairy waste, other raw materials are processed. Over the 12-year period covered in the table, 24 projects were added and 14 were shut down. In the spring of 2018, 20 digesters were in operation, with the oldest four having begun operations in 2004 and the newest three added in 2018. As of this writing, six digesters were scheduled to open in 2019 (EPA 2018a). This data indicates that, despite government support, digesters in California have not yet experienced widespread adoption.

Policies and programs

A recent California policy change enhanced the potential payoff for dairy digesters that produce renewable natural gas. The California Low Carbon Fuel Standard (LCFS) Program awards tradable credits to producers of eligible low-carbon transportation fuels. In December 2015, CARB announced that California would begin to allow LCFS credits for production of vehicle fuel derived from biogas that counts toward avoided dairy methane emissions, using the ARB Livestock Offset Protocol (California Bioenergy 2015; CARB 2018a). Prior to this policy change, avoided emissions from dairy digesters could be used as carbon credits under the state's cap-and-trade program, which were worth only about one-tenth as much as the LCFS credits.

During the last few years, much government support — such as subsidies for project development efforts — has been directed to projects that produce vehicle fuel, mainly renewable compressed natural gas. In 2017, the CDFA's Dairy Digester Research and Development Program — a partnership of state, federal and local agencies — awarded financial support, totaling $35 million, to 18 digester projects. Eleven of the 18 projects focus on producing renewable compressed natural gas, with the rest of the projects primarily used to power an ethanol refinery (CDFA 2018).

In the 2017 budget year, money available in a dairy digester fund financed by the Greenhouse Gas Reduction Fund totaled $99 million, and of this amount, over $60 million will be disbursed by the Dairy Digester Research and Development Program to support the construction of dairy digesters producing vehicle fuel. Several projects producing renewable compressed natural gas at commercial scale will come online soon. This is an important development for the state's greenhouse-gas mitigation efforts and for the California dairy industry, which must comply with mandates for manure-related methane reductions.

Economics of renewable natural gas production

Numerous studies have evaluated digester investments, but the following discussion focuses on recent CARB estimates of the costs and revenues associated with producing renewable natural gas at a cluster-based locally centralized digester (CARB 2017). Lee and Sumner (2014) have reviewed costs and returns for digester projects through 2014. Environmental Science Associates (2011) and the California Dairy Campaign (2013) have reviewed costs associated with a centralized digester system. CARB bases its estimates on a stylized 2,000-cow dairy farm; the farm participates in a cluster that operates a locally centralized digester system. CARB assumes that the San Joaquin Valley would contain 55 such local clusters, handling manure collected from a total of 1.05 million cows. This number represents almost 60% of the milk cows in California and almost two-thirds of the dairy cows in the San Joaquin Valley.

Table 2 summarizes CARB's estimates of each farm's share of the capital cost of building the locally centralized digester — a cost shared among the cluster's members — and each farm's annual flow of costs and revenues from digester operation. We begin our discussion by reviewing these cost and revenue figures.

Costs

The capital cost for the locally centralized digester system specified by CARB is about $4.8 million for a typical farm. Capital cost is the total of the one-time expenses when the project is initiated, which include collective costs for building the digester itself, pipeline construction, manure transportation equipment and interconnection (costs to connect to and inject renewable natural gas into the main utility pipeline). Capital cost also includes the investments that each farm must make to convert to a dry-scrape system that will allow dry manure to be collected and transported to the central location. (This article evaluates the accounting and financial data that CARB presents for a system of this kind. We do not attempt to critique or evaluate other implications of the system, such as the relative costs or benefits of handling the effluent at the central location rather than at each farm.)

In addition to capital cost, each farm participating in a digester system would be responsible for a share, totaling about $588,000, of the annual expenses associated with operating and maintaining the system (CARB 2017). Shifting to the locally centralized digester system would likely allow farms to save some of the costs associated with traditional manure handling. Although we do not have data that specifically breaks down these costs, dairy cost studies available from the CDFA indicate that average manure handling costs for large San Joaquin Valley dairies (CDFA 2017) are about $14,000 per year.

To appreciate the financial implications for a typical dairy farm participating in a locally centralized digester system, let us view these capital and operating costs in the context of the typical farm's milk revenue. Using a 2017 average milk price of $16.50 per hundredweight (100 pounds), a farm with 2,000 cows producing 230 hundredweight per cow per year (the average in the San Joaquin Valley) would have annual milk revenue of almost $7.6 million (CDFA 2017). Thus, the digester's operating costs are close to 8% of milk revenue — equal to the farm's costs for hired labor and larger than any other operating cost except for feed and replacement cows (CDFA 2017). In 2017, based on CDFA's cost estimates, average milk production and market prices, net revenue calculated at the typical dairy in the southern San Joaquin Valley amounted to zero.

The capital cost of a centralized digester — $4.8 million — likely represents the largest single investment on a dairy farm with 2,000 cows. For comparison, if cows cost $2,000 each, the farm's investment in 2,000 cows is $4 million. Thus, the typical farm will invest more in the centralized digester than in the establishment of its entire herd.

Revenues

Revenue from the centralized digester includes sales of renewable natural gas and income expected from biofuel credit programs created by the California and U.S. governments. Revenue from credits created by California policy depends on specific features of the California LCFS program. Revenue from federal credits depends on features of the Renewable Fuel Standard

TABLE 2. Costs, revenues and net present value of a digester project producing pipeline-injectable natural gas, per participating farm

Costs	Capital cost	Annual O&M cost
Scrape conversion	$696,000	$21,000
Digester	$2,905,000	$174,000
Pipeline (low pressure)	$75,000	$4,000
Pipeline (transmission)	$104,000	$5,000
Low NOx truck purchase	$140,000	—
Manure hauling	—	$95,000
Interconnection	$849,000	$30,000
Upgrading the biogas*	—	$258,000
CNG station (small fleet)	$23,000	$2,000
Total cost	**$4,792,000**	**$588,000 ‡**
Revenue		**Annual revenue**
Fuel sales ($3.46/1,000 ft^3)	—	$149,000
RINs ($1.85/credit)	—	$1,060,000
LCFS credits ($100/credit)	—	$865,000
Total revenue	—	$2,074,000
Net present value†	**—**	**$6,203,000**

* Capital cost for upgrading biogas is embedded in the O&M cost.
† Present value calculations assume a 10-year life for the project, a 7% interest rate for amortizing capital cost and a 5% discount rate for future revenues.
‡ Total differs from sum of values above due to rounding.
Source: CARB (2017), Table 14 of Appendix F.

A manure digester at a dairy in Sacramento County. Because a planned system of centralized digesters in the San Joaquin Valley relies heavily on policy-dependent revenue streams, the system's economic viability could be vulnerable to changing political conditions.

(RFS) — also referred to as RIN credits, where "RIN" stands for "renewable identification number."

The LCFS is one of the main greenhouse-gas reduction measures adopted to implement AB 32, the California Global Warming Solutions Act of 2006. Under the LCFS, each eligible transportation fuel is assigned a carbon intensity that indicates the fuel's estimated greenhouse-gas emissions over its life cycle — including extraction, production, transportation and consumption. LCFS credits or deficits are calculated based on each fuel's carbon-equivalent intensity (CARB 2016; CARB 2018a).

The RFS program is a national policy that requires refiners to replace a certain share of petroleum-based transportation fuel, heating oil or jet fuel with renewable fuels (EPA 2017b). Under the program, producers of renewable fuel — such as operators of centralized digesters — earn RIN credits, which can be sold to refiners to satisfy their RFS requirements (EPA 2017b). The RFS program has been controversial. It is potentially subject to substantial revision, or even elimination, by congressional or administrative action. As of this writing, in November 2018, the program is operating as usual — while the U.S. Environmental Protection Agency and congressional leaders debate whether the program will continue and, if so, in what form.

As shown in table 2, CARB estimates that annual per-farm revenues for commercial gas sales are $149,000 (at $3.46/1,000 cubic feet). For federal RIN sales (at $1.85/77,000 BTU), CARB assumes per-farm revenue of $1,060,000. The RIN price and the associated revenue are influenced by the U.S. prices of petroleum and corn, as well as the Brazilian price of sugar cane. All these prices contribute to the price of ethanol — the dominant renewable fuel that qualifies under the program.

CARB (2017) assumes that California's LCFS credits will contribute revenue of $865,000 (assuming $100 per metric ton of CO_2e) to the typical farm. (As a unit of measurement, CO_2e provides a common denominator for the global warming potential of different greenhouse gases. Methane is a more potent greenhouse gas than carbon dioxide; 1 metric ton of methane is equivalent to 25 metric tons of CO_2.) The value of these credits depends on the equilibrium market price of emission credits in California and hence on broader supply and demand for emission credits from many sources of reduction of greenhouse gases.

Of the $2.074 million in total annual projected revenue for the digester, only 7% comes from selling renewable natural gas in commercial markets. About 93% of the projected revenue comes from selling government-created environmental credits. CARB estimated revenues from government programs based on the assumption that "current" prices of credits — that is, prices in April 2016, when the CARB study was initiated — were the best predictor of prices over the life of a digester project.

Calculating net present value

Any investment project must be evaluated in terms of the time paths of revenues (and other benefits) generated and expenses (and other costs) incurred during the life span of the project's capital inputs. When a project spreads over multiple time periods, it is often evaluated through a calculation of net present value. A present value of a future stream of receipts or payments over time uses an interest rate or time value of money to convert each transaction into its current equivalent; the net present value of a project is the difference between the present value of inflows and outflows over the life of the project. If the net present value is positive, the project earns a positive return above the threshold rate of return for the funds invested.

CARB's analysis of the digester project assumes a 10-year horizon for the effective economic life, including depreciation and obsolescence, of all the digester capital inputs. The net present value calculation for the digester project described above uses an amortization rate (assumed to be 7%) to reflect the interest paid (or foregone) on the invested capital. A 5% discount rate is used to bring the stream of net revenue over the future 10 years back to present-value terms so that it can be compared to the up-front investment. The discount rate reflects the time value of money and thus the value of foregone future investments.

Under these assumptions, CARB finds a net present value of $6.2 million for each farm's digester investment (CARB 2017). Thus, despite large capital investments and substantial annual operating costs, projected revenues generate a very large gain for investors in a local centralized digester. The next section considers more thoroughly the assumptions that underlie this projected profitability.

Alternative policy scenarios

As noted above, projected digester revenue depends primarily on California LCFS credits and federal RIN credits. This dependence means that changes in state or federal policies on energy or environment issues — or even changes in the technical details of program operation — could substantially alter economic calculations pertaining to digester investment. Because digesters rely on income generated by policy-created assets, government policy risk is inherent in their revenue and profitability.

Because of the design of the LCFS and RIN programs, credit prices vary with specific market conditions such as the price of corn or oil. Moreover, the specifics of the policies change in response to political forces — and these changes can in turn affect relevant markets. We consider here several LCFS policy risk scenarios that are reflected in alternative LCFS credit prices of $120, $100 and $75 per metric ton of CO_2e.

Since 2013, the market price of LCFS credits has fluctuated between $20 and $125 per metric ton of CO_2e. Recent prices have been relatively high, exceeding $100 per metric ton (CARB 2018b). Smith (2016) describes a huge jump in the price of LCFS credits in 2016 — after CARB, in 2015, changed a technical detail in the LCFS formula. In light of fluctuating historical prices and the potential for further changes to the formula, we chose a range from $120 to $75 to represent the upside and downside market risk relative to CARB's reference price for LCFS credits — $100 per metric ton.

Historical RIN prices have also fluctuated. Out of four categories of RINs, renewable natural gas belongs to the highest-priced category (known as D3 RIN, or cellulosic RIN). The credit price for D3 RIN is determined by adding a cellulosic waiver credit, which is set annually by formula pricing (Sheehy and Rosenfeld 2017), to the market-determined price of D5 RINs. The cellulosic waiver credit represents the lion's share of the value of D3 RINs. Over the last 4 years, the D5 RIN price has fallen in the range of 60 to 80 cents — whereas the cellulosic waiver credit has ranged between 64 cents and $2 (64 cents in 2015, $1.33 in 2016, $2 in 2017 and $1.96 in 2018) (EPA 2018b).

The major risk associated with the value of RIN credits lies in the risk that features of renewable fuel standards will change, perhaps substantially. The RFS program, which authorizes RIN credits, is more politically vulnerable than the LCFS program. The risks potentially include elimination of the program (Wall Street Journal 2018). To account for recent trends in pricing of credits and for uncertainty surrounding the policies that will affect future RIN credits, we develop scenarios in which revenue associated with RIN credits increases by 25% — or decreases by 25%, 50%, 75% or 100% — from the baseline assumed by CARB. Digesters would receive no RIN revenue if the federal RFS program were eliminated or if it were changed so that benefits for the California manure digester program were removed.

Finally, our revenue scenarios are developed under two overarching California LCFS credit regimes, which we call "pre-regulation" and "post-regulation." These two regimes are distinct periods falling before and after mandatory regulations are fully implemented — which, under SB 1383, is scheduled for 2024. During the pre-regulation period, methane reduction is not mandatory — and avoided methane emissions are thus credited for LCFS credit calculations. During the post-regulation period, when methane reduction is mandatory, avoided methane emissions no longer earn LCFS credits. In other words, credits are awarded against a baseline — and the baseline changes once mandatory regulations take effect.

According to CARB data, the applicable carbon intensity of manure-based biofuel would increase after regulation to 13 gCO_2e per megajoule from −276 gCO_2e per megajoule. This increase in carbon intensity would lower LCFS credit revenue for manure-based biofuel to $110,000 per year from $865,000 per year, meaning that

TABLE 3. Net present values ($ million) corresponding to alternative LCFS price and RIN revenue scenarios under pre- and post-regulation conditions

	LCFS credit price					
	$120	$100	$75	$120	$100	$75
	Pre-regulation (CI = −276 gCO_2/MJ)			*Post-regulation (CI = 13 gCO_2/MJ)*		
RIN revenue 125%*	$9.59	$8.25	$6.58	$2.61	$2.41	$2.21
RIN revenue 100%	$7.54	$6.20	$4.54	$0.55	$0.37	$0.16
RIN revenue 75%	$5.50	$4.16	$2.49	−$1.50	−$1.67	−$1.89
RIN revenue 50%	$3.45	$2.11	$0.44	−$3.55	−$3.72	−$3.93
RIN revenue 25%	$1.40	$0.07	−$1.60	−$5.59	−$5.76	−$5.98
RIN completely removed	−$0.64	−$1.98	−$3.65	−$7.64	−$7.81	−$8.02

* Proportion of CARB's reference RIN revenue.
Source: Authors' calculation.

manure-based fuels would earn only 13% of the LCFS credits that they earned before regulation (CARB 2017). According to CARB (2017), projects that begin to operate before reduced methane emissions become mandatory may apply the pre-regulation carbon intensity to their credit calculations for the full 10-year life of the digester system (CARB 2017, Appendix F, 11).

Table 3 shows the effects on net present value that result from several scenarios involving potential LCFS credit prices and RIN revenues. In order to facilitate comparisons, we highlight a reference scenario under which the LCFS credit price is $100, RIN revenue remains at 100% of its currently assumed level and net present value is $6.2 million. Under the pre-regulation regime, almost all scenarios generate positive net present values. The exceptions are scenarios that assume either elimination of RIN credits or a 75% reduction in revenue from both RIN and LCFS credits. The post-regulation regime scenarios, however, with their large reductions in LCFS revenue, yield much lower and often negative net present values. Under this regime, positive net present values occur only when RIN revenue is at least equal to the reference level (100%) assumed by CARB. Under the post-regulation regime, RIN income becomes even more crucial to establishing positive net present value, highlighting the significance of RFS policy risk.

Policy changes can flow from political realignments or from new technical information. Given the complexity of the LCFS and RFS policies, even small technical adjustments to regulations can have major effects on policy-generated revenues. In 2016, for example, California adjusted certain details of LCFS calculations — details involving indirect land-use impacts and the carbon intensity of crop-based biofuel. This adjustment resulted in a higher implied carbon intensity for corn-based ethanol and thus a reduction in LCFS credits per unit of ethanol (Smith 2016). This change in a technical detail created a market shortage in the supply of LCFS credits. In the first quarter of 2016, the shortage caused the market price for credits to increase to as much as $123 per ton from about $20 per ton (Smith 2016). Such technical adjustments can cause decreases in price just as easily as increases in price. The adjustments may involve seemingly minor details, unrelated to digesters or even to conditions in California.

Federal RFS policy, which creates value for RINs, has come under increasing political pressure as ethanol's environmental contributions have been questioned (Smith 2016). In the long term, RIN credits are much more critical than LCFS credits to digester revenue. If only 13% of pre-regulation LCFS credits are available to a digester that comes online after implementation of California's mandatory regulation of dairy methane, revenue from federal RIN credits would account for more than 80% of the digester's revenue. Therefore, the economic viability of newly built digesters under the post-regulation regime depends crucially on revenue from the federal program.

For locally centralized digester systems, economic viability clearly requires that certain policies remain largely unchanged. Investment in digester systems therefore depends on investor willingness to accept policy risk as a major economic consideration. In that context, if the California government wants to encourage investment in such projects, it might wish to consider establishing government assurances, or a government-backed insurance program, to cover losses associated with possible changes in state or federal policies.

A final investment issue concerns market-based uncertainty related to the scale of digester projects and the future economic health of the dairy industry in the San Joaquin Valley. Locally centralized digester systems producing pipeline-injectable biogas require a large up-front capital investment, which implies substantial scale economies. In California, however, the numbers of dairy cows — after rising rapidly until 2007 — have been declining gradually for more than a decade, as other dairy states have become more efficient (CDFA 2016). If dairy operations face additional costs due to implementation of greenhouse-gas rules or other regulations, further decreases in the number of cows are likely. If neighboring farms that co-invest in a digester project exit, the remaining farms will face higher costs. In-depth analysis of a potential digester investment must incorporate the probability that neighbors and their cows may leave, causing a risk that the per-farm costs assumed in table 2 may be too low.

As noted above, our analysis is limited to the emission reduction pathway, which assumes that manure is scraped and hauled to a locally centralized facility to produce pipeline-quality natural gas. Further research should examine alternative pathways, such as on-farm digesters connected to a central facility for compressed natural gas, where biogas is conditioned and upgraded. Such research would provide a fuller assessment of the potential economic consequences of the new policies.

Policy risk threatens investment

Developing cluster-based, anaerobic, locally centralized digester systems — systems that produce renewable natural gas — may offer the California dairy industry an economically viable way to comply with mandatory methane regulations. Investment in such projects, however, requires a large commitment of capital compared to other dairy investments. Investing in a digester also involves considerable uncertainty — uncertainty that falls outside the variability in milk and feed markets that farmers have long been familiar with. Issues outside the farmer's control that affect a digester's payoff include unfamiliar technical specifications and operational details, variable energy prices and unexpected shifts in, or rapidly evolving, state and national regulations and policies. For any investment whose economic outcome depends primarily on continuation of favorable government policies, long-term assurance of and clarity regarding policy are crucial. A policy under which the government assumes some of the downside policy risk could enhance confidence in revenue stability.

Under California's evolving methane regulations, emissions from manure handling must be reduced. Any net cost involved in achieving low greenhouse-gas emissions can be considered part of the routine cost of milk production and processing in California. If costs are added to those already borne by dairy farms and processors, the California dairy industry will find it more challenging to maintain national and global competitiveness. Unless handled carefully, new methane regulations could erode the economic position of dairy farms and processors in California. If that were to happen, we would expect additional exits from the industry or relocation out of California, resulting in reduced dairy-related economic activity, especially in the San Joaquin Valley (Medellín-Azuara et al. 2018; Sumner et al. 2015). The potential for such a downward spiral deserves careful consideration.

We document the crucial role of policy-generated income and risks inherent in such income. Dairy digesters can survive or even thrive in California if policy uncertainty is mitigated and policy-generated revenue flows are assured. Without such assurances, or some other source of revenue or government funds to cover invested capital, digesters may be too risky to warrant investment. If they are not carefully implemented, California regulations will fail to reduce global greenhouse-gas emissions — indeed, they will simply shift methane emissions to other locations while also eroding the economic viability of the California dairy industry. CA

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RESEARCH ARTICLE

Closing the extension gap: Information and communication technology in sustainable agriculture

Survey results suggest that time constraints, technical complexity and the potential for misinformation are barriers to the adoption of information and communication technology tools among extension professionals.

by Mark Lubell and Neil McRoberts

Abstract

As the information revolution sweeps through the agricultural sector, extension professionals may be lagging behind their clients in the use of information and communication technology (ICT) such as social media, which could be a valuable tool for outreach and education. We surveyed sustainable agriculture stakeholders in California — extension professionals, county agricultural commissioners, and members of farm bureaus and producer groups — to measure their ICT behavior and attitudes. Drawing on diffusion of innovation theory, we characterized the innovation attributes of ICT that may influence the adoption and use of new technology among extension professionals. We also studied their demographic characteristics to establish whether there was a connection with ICT use. The main perceived benefit of ICT was that it can quickly reach larger, more diverse and more distant audiences. The perceived challenges included lack of professional support, the potential for misinformation on social media platforms, and the time requirements and technical complexity of technology use. Extension professionals experienced these challenges more than other sustainable agriculture stakeholders, creating a technology gap between extension professionals and their clientele. An ICT community of practice and clear organizational guidelines for measuring and reporting performance relating to ICT might help extension professionals close the gap.

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Farmers and other agricultural stakeholders are experimenting with many types of information and communication technology (ICT) such as websites, blogs, social media and mobile decision-support applications. As data scientists integrate ICT with "big" data, farmers can downscale diverse sets of information for local decision-making and upscale local data to see emergent patterns at multiple scales. Social media tools allow extension professionals, farmers and other agricultural stakeholders to communicate in new ways about the broad range of issues affecting agroecological systems. The increasing use of ICT in agriculture has engendered a significant debate about its benefits for achieving extension goals relative to its potential risks and costs.

This paper empirically examines ICT use among extension professionals working on sustainable agriculture in California. We broadly define "extension professionals" as professionals engaged in agriculture outreach and extension, either based at a university (e.g., Cooperative Extension specialists, university faculty, county agents) or elsewhere throughout

New research findings on agricultural stakeholders' use of information and communication technology (ICT) — websites, blogs, social media and mobile apps — indicate that UC employees used fewer ICT platforms and used social media less frequently than other professionals in the field of agriculture outreach and extension.

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The traditional top-down model of delivering land-grant university research to local clientele is becoming obsolete, especially when resources are thin. New ICTs are potentially important tools in this endeavor, especially when used to complement other methods of outreach and education.

the food system and agricultural knowledge networks (e.g., consultants, members of nongovernmental organizations such as county farm bureaus and producer associations). We particularly emphasize the role of social media platforms such as Twitter, Facebook and LinkedIn as innovative extension tools for building knowledge networks, coordination, communication, outreach and education.

We draw on diffusion of innovation theory as a framework that can integrate many elements of the debate about the benefits and risks of ICT (Feder and Umali 1993; Prokopy et al. 2008; Rogers 2010). Diffusion of innovation theory suggests that ICT adoption depends on how extension professionals perceive the attributes of this innovative technology, such as its relative advantage over other extension tools and its complexity. We also examine how demographic characteristics of extension professionals influence ICT adoption. Our analysis sheds light on the potential technology gap, hinted at by extant research, between extension professionals' use of ICT and the general public's, and possibly agricultural clientele's, greater use of ICT. Developing policy recommendations to improve the appropriate use of ICT requires identifying the critical barriers to ICT adoption among extension professionals.

Our research has implications for broader ideas about how to adapt extension systems to the new realities of agricultural knowledge networks and innovation systems (Klerkx et al. 2010; Klerkx et al. 2015; Klerkx and Proctor 2013; Lubell et al. 2014). Modern agricultural knowledge networks are distributed systems, where relevant information is developed and communicated by a wide range of stakeholders.

The traditional top-down model of delivering land-grant university research to local clientele is becoming obsolete, especially when resources are thin (Carr and Wilkinson 2005). It must be complemented by a more bottom-up model, where in addition to developing and broadcasting new knowledge, land-grant universities and other extension organizations must build innovation systems that coordinate knowledge networks among different stakeholders (Lubell et al. 2014). Such networks seek to synergistically combine social, technical and experiential learning. New ICTs are potentially important tools in this endeavor, especially when used to complement other methods of outreach and education. The results of this paper enhance the evidence base for this endeavor.

An extension technology gap?

The information technology revolution has transformed the way that people access information and build social connections (Barabasi 2003; Rainie and Wellman 2012; Watts 2004) across the globe. The latest survey results from the Pew Research Center (2016) estimated that the percentage of U.S. citizens using at least one social media site increased from 5% in 2005 to 69% in 2016. Social media use was more frequent among women and individuals in higher education and income categories. In 2016, Facebook had the highest market share (68%), followed by Instagram (28%), Pinterest (26%), LinkedIn (25%) and Twitter (21%).

Farmers are increasingly connected but lag behind the general population. USDA NASS (2017) estimated that in 2017 more than 70% of farmers in the United States had computer and internet access and 47% used computers for farm business. Computer and internet usage was higher among wealthy farmers. A study in the Pacific Northwest (Guenther and Swann 2011) found that potato growers used popular ICT platforms as frequently as college students — 93% of growers used email compared with 97% of students; 97% of growers used text messages compared with 94% of students; 70% of growers used Facebook compared with 73% of students; and 90% of growers used YouTube compared with 91% of students — and growers overall used 3.5 more varieties of technology than college students. In developing countries, mobile phone technology continues to expand and provides a crucial information and networking resource for rural agricultural populations (Aker 2011; Matous et al. 2011; Matous et al. 2015; Matous 2017).

Despite some evidence that extension clientele are using ICT at rates approaching those of the general population, extension professionals may be lagging behind both groups. Gharis et al. (2014) reported that among participants in a Natural Resources Conservation Service webinar, 53% used Facebook

Despite some evidence that extension clientele are using ICT at rates approaching those of the general population, extension professionals may be lagging behind both groups.

and 10% used Twitter. O'Neill et al. (2011) found that the proportion of members of the financial services community of practice for e-Extension using Facebook (42%) or Twitter (7%) daily is far less than the general population. While the existing research hints at a potential technology gap in extension professionals' use of ICT, much more research is needed to document and explain ICT adoption and use within agricultural systems.

The potential gap in extension professionals' use of ICT reflects a lively ongoing debate about the costs, benefits, barriers and risks of ICT for agriculture (Fuess 2011; Gharis et al. 2014; Newbury et al. 2014; Seger 2011). On the benefits side, ICT may provide access to information, coordination, job opportunities, social networks and improved services (Aker 2011). Extension professionals expect ICT to create a snowball effect (Cornelisse et al. 2011), with information more quickly reaching a larger and more diverse audience than in-person communication methods like workshops and field meetings (Gadino et al. 2016). The benefits may include the integration of real-time information into mobile applications or websites to provide decision support, linking daily agricultural decisions to economic and agro-ecological processes at multiple scales.

Realizing these benefits requires overcoming many potential risks, barriers and costs. Gadino et al. (2016) highlighted the importance of linking traditional in-person methods with digital technology and the time required to update ICT with new and real-time information. Newbury et al. (2014) identified the barriers as lack of training, concern about information control and time availability. Gharis et al. (2014) emphasize lack of professional acceptance by colleagues as a barrier to innovation, which is linked to the capacity to measure effectiveness. O'Neill et al. (2011) pointed out the need for organizational procedures; only 29% said their institutions had guidelines for reporting, and only 22% of their respondents reported their own social media outreach activities to their extension administration. There was a notable amount of uncertainty — 27% of nonreporters said they did not know how to use social media, and 38% did not know if their institution had guidelines.

Diffusion of innovation theory

Existing research lacks a theoretical framework to integrate the diverse terms of the debate about ICT adoption among extension professionals. Diffusion of innovation theory, which examines how innovations spread through a population of users, provides such a framework. It has been an enduring research topic in agricultural decision-making for more than a century (Feder and Umali 1993; Prokopy et al. 2008; Rogers 2010; Ryan and Gross 1947; Wejnert 2002). A central argument of diffusion theory is that the likelihood of an innovation being adopted is related to the following attributes of the innovation: relative advantage, compatibility, complexity, trialability and observability. We used these attributes to frame our research hypotheses.

"Relative advantage" refers to the innovation's potential benefits and opportunities relative to other extension tools. For ICT, the most frequently discussed advantages are its capacity to reach larger, more diverse and more geographically dispersed audiences (Aker 2011; Cornelisse et al. 2011; Gadino et al. 2016). Also, ICT can quickly deliver new information, potentially in real time with linkages to large-scale data. ICT may also provide support for on-the-ground decisions, for example, about agriculture management, or for coordinating the activities of extension professionals.

"Compatibility" is the extent to which the innovation is compatible with professional and social norms. For extension, an important norm is delivering scientifically valid and neutral information to support decision-making and stakeholder dialogue. Especially with the everyday mention of "fake news" and "internet trolls," extension professionals worry that social media may facilitate the spread of misinformation and provide an avenue for unreasonable individuals to corrode civic dialogue. In addition, many extension professionals feel that relative to more traditional outreach and publication strategies, there is a lack of professional incentives and peer recognition for the use of ICT.

"Complexity" refers to the difficulties of integrating the innovation. In terms of the ICT debate, not all extension professionals have the technical knowledge to effectively use social media platforms or effectively integrate communication across multiple platforms. It may take too much time to learn how to use social media and maintain an active web presence. These complexities are exacerbated by a lack of widely recognized best practices about how to effectively craft social media communication.

"Observability" and "trialability" refer to the extent to which the innovation's effectiveness can be observed and tracked. There is a lack of clarity about how to track the effectiveness of ICT, for example, observing who accesses and uptakes information posted on Facebook or Twitter (Gharis and Hightower 2017). This includes the use of altmetrics, since there is no universally accepted method of measuring social media effectiveness and no clear policies from the University of California, UC Agriculture and Natural Resources, or other organizations. Furthermore, it is more difficult to control access to or target the audience for social media information with the same precision as in-person strategies aimed at particular constituencies.

ICT use study

We studied ICT use among extension professionals involved in sustainable agriculture in California. An empirical study, it analyzed whether ICT adoption and use was affected by perceptions about ICT (the innovation attributes described above) and the professional demographics of the individual user. The data came from 661 respondents to a statewide survey fielded between May and July 2016, which achieved an overall response rate of 28% (see technical appendix, http://ucanr.edu/u.cfm?id=214).

In addition to UC Cooperative Extension professionals, the survey included people from organizations that are part of the knowledge network engaged in outreach, education and communication: producer groups, nongovernmental organizations, consultants, resource conservation districts, government agencies and others. While some respondents did manage farms, we were not targeting farmers but rather those who develop and deliver information to farmers. It would be useful for future research to extend the survey to farmer populations, specific consultant groups such as pest control advisors, and agricultural knowledge networks in other countries and U.S. states.

Using the framework of the diffusion of innovation theory, our analysis tested the following hypotheses: extension professionals who perceive a greater relative advantage are more likely to adopt ICT; less likely to adopt ICT are extension professionals who perceive ICT as incompatible with their values and social norms, extension professionals who perceive ICT as too complex or time consuming and extension professionals who are uncertain about how to measure ICT effectiveness or strategically target audiences.

In addition, we tested how demographic factors may be linked to ICT adoption, with the expectation that the patterns would be similar to the patterns in the general population. To pursue the possibility of an extension gap, we tested whether UC employees had a lower ICT adoption rate relative to other types of extension professionals.

Survey, analysis models

We tested our hypotheses by first constructing dependent variables for the number of ICT platforms used and the frequency of social media use. The survey assessed ICT adoption by asking respondents if they used blogs, websites, email, mobile applications, Facebook, Twitter, Pinterest, Instagram or LinkedIn to communicate or learn about sustainable agriculture. We constructed a yes/no variable for each ICT platform.

To zero in on the frequency of use for just the social media platforms (Facebook, Twitter, Pinterest, Instagram and LinkedIn), we followed the Pew Internet Survey in establishing the following categories of use: several times a day, once a day, a few days a week, every few weeks, or less often. Importantly, the focus was on using social media for professional communication about sustainable agriculture, not personal use of social media.

To analyze how perceptions about the attributes of ICT are related to ICT behavior, we constructed a social media frequency scale that calculated the average frequency of social media use across all five platforms, plus an "other social media" category. The scale ranged from 1 = do not use any social media to 6 = use all social media several times per day, with mean = 2.01. To calculate the number of total ICT platforms used, not just the social media platforms, we summed the number of platforms respondents checked; the numbers ranged from 0 to 9, with mean = 3.81.

We then estimated multivariate models with social media frequency (ordinary least squares regression) and total number of ICT platforms (Poisson regression) as dependent variables, and the four attributes of innovations (relative advantage, compatibility, complexity, and observability and trialability) as independent variables (see technical appendix for survey wording and descriptive results for innovation attributes). Respondents' perception of social media's relative advantage was measured by averaging their responses to six statements related to its capacity to reach audiences and help extension professionals coordinate professional activities. These statements form a reliable scale (Cronbach's alpha = 0.84) ranging from 1 = strongly disagree on all statements to 5 = strongly agree on all statements, with mean = 3.71.

Respondents' perception of compatibility was measured in their responses to four statements: social media risks spreading fake news, there are positive incentives for its use, most colleagues use it, and it involves too much interaction with trolls. The response options did not form a reliable scale, so we included each statement as a separate variable in the analysis.

Perception of social media complexity was measured in responses to these four statements: it takes too much time, it's technically difficult to use, best practices are well known, and the large number of platforms is confusing. Again, the response options did not form a reliable scale, so we included each statement as a separate variable in the analysis.

Lastly, respondents' perception of the measurability (or observability and trialability) of social media's effectiveness was assessed. One statement suggested it was easy to measure effectiveness; the other statement suggested it was easy to identify appropriate audiences for social media. Responses were averaged into a reliable scale (Cronbach's alpha = 0.74) that ranged from 1 = strongly disagree on both statements to 5 = strongly agree on both statements, with mean = 2.32.

To test the effect on ICT adoption of users' demographic characteristics, we used the same dependent variables as described above, and we estimated the same models using the following demographic independent variables: sustainability attitude (five-point Likert scale; 1 = sustainability deserves much less emphasis, 5 = sustainability deserves much more emphasis), age (mean = 53.2), income (eight-category scale ranging from less than $25,000 to $200,000 or more household income before taxes in last 12 months; modal category was $100,000 to $149,000), UC system (dummy variable indicating employees of UC or UC Agriculture and Natural Resources), male (dummy variable; 1 = male, 0 = female/other) and education (seven-category scale ranging from did not graduate high school to advanced degree; modal category was advanced degree — M.A., M.D., Ph.D.).

Patterns of ICT use

Figure 1 reports the overall adoption rates for the ICT platforms. In decreasing order of use, email was used by 92% of the respondents, followed by websites (80%) and Facebook (58%), with Instagram and Pinterest having the lowest adoption rates. While the results for the most popular platforms echo the results for the general population, Twitter (37%) and LinkedIn (51%) were used relatively more by extension professionals because they are specifically intended for information dissemination and professional networking.

Figure 2 reports the average temporal frequency indicated by each respondent for using just social media. For the general population, Pew reports that 55% of Facebook users and 23% of Twitter users access their accounts several times per day. In contrast, our sustainable agriculture stakeholders in California access Twitter and Facebook at the lower rates of once a day or a few days per week. The lower frequency of use for LinkedIn most likely reflects that the content (professional profiles and events) changes more slowly than the events communicated on Twitter and Facebook.

Figure 3 plots the coefficients (see technical appendix for full model results) from an ordinary least squares regression model for social media frequency and a Poisson model for number of ICT platforms (a count variable), with the variables arranged in order of decreasing magnitude from the social media frequency model. Relative advantage had the strongest positive relationship with both the frequency of social media use and number of ICT platforms. Respondents who thought most of their colleagues used social media also used more ICT platforms, more frequently.

Technical difficulty and concern about trolls had negative effects in both models. Interestingly, the attributes of innovations were less important for the number of ICT platforms used than for the frequency of social media use, where the capacity to measure effectiveness and identify the audience had a positive influence and lack of time and concern about fake news had strong negative effects. The existence of professional incentives, confusion about the number of platforms and knowledge about best practices were unimportant in both models.

Figure 4 suggests some demographic variables behaved in ways consistent with the general population: ICT and social media use was higher among female, younger and wealthier respondents. The results also corroborated the technology gap described earlier: UC employees used fewer ICT platforms and used social media less frequently than other respondents. More educated respondents also used social media less frequently, which contrasts to the general population, where educational levels are positively correlated with social media use.

In the context of professional extension activities, UC system employees or those with advanced degrees may be stressed for time, perceive social media as incompatible with norms of scientific knowledge sharing or lack professional incentives. The breakdown of UC respondents was 35% campus faculty, 13% Cooperative Extension specialist, 22% Cooperative Extension county advisor, 6% other academic title, 10% student/postdoctoral scholar, 11% staff and 3% other.

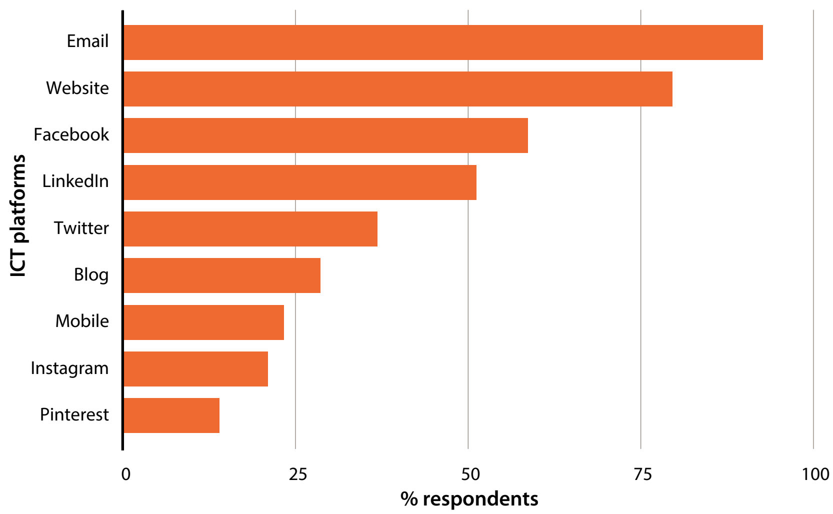

FIG. 1. Which of the following information and communication technologies, if any, do you use in your professional responsibilities?

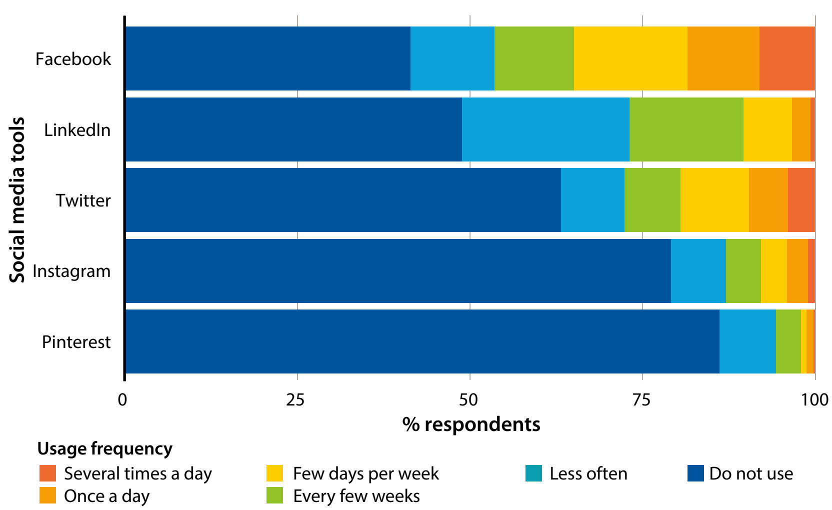

FIG. 2. How frequently do you use the following social media tools to communicate or learn about sustainable agriculture?

Communities of practice

Sustainable agriculture stakeholders in both developed and developing countries are quickly catching up to the information revolution that has transformed society in the 21st century. Our results confirmed the usefulness of diffusion of information theory, which frames the debate about the benefits and risks of ICT in terms of innovation attributes related to relative advantage, compatibility, complexity, observability and trialability. Extension professionals clearly recognized the relative advantages for ICT in terms of quickly communicating with a more diverse and distant audience, but with less potential to coordinate on-the-ground activities. Extension professionals are more likely to capitalize on these relative advantages if their colleagues are also using ICT, and they have good tools for measuring effectiveness. The most important barriers for widespread adoption of ICT were time constraints, technical complexity and incompatibility between norms of scientific discourse and the reality of trolls and misinformation on the internet.

These results support some concrete recommendations for organizations seeking to increase the use of ICT and make it more effective for extension professionals. Resources could be invested in developing a community of practice for aspiring ICT users interested in using ICT for outreach, with leadership from extension professionals with an established reputation for successful innovation. Communities of practice are one of the organizational concepts in e-eXtension, and are defined as informal networks of professionals with a common goal who regularly interact to share information and expertise (Wenger and Snyder 2000). They can help creatively solve problems, transfer knowledge and develop professional skills and are effective where the network of individuals is distributed across many administrative units or system components, as is the case with extension professionals experimenting with ICT.

A sponsoring organization can help foster a community of practice by identifying potential members, providing organizational infrastructure for interaction and measuring effectiveness with appropriate metrics. Such a community of practice should document the potential advantages of ICT and provide information about best practices. It would increase awareness about how many extension professionals are using ICT, which would help create a community norm. The community of practice should include a diverse set of stakeholders, including digital technology specialists from outside of agriculture who are knowledgeable about different types of tools, altmetrics and social media strategies that are effective in digital communication.

It is also important for agricultural extension organizations, including land-grant universities, to establish clear guidelines for recognizing the value of ICT as an extension tool that complements traditional communication strategies and ways of extending knowledge. If extension professionals know what counts in terms of documenting their professional activities for job performance evaluation, they are less likely to be confused and view ICT as a risky endeavor. Developing such guidelines would benefit from consultation by

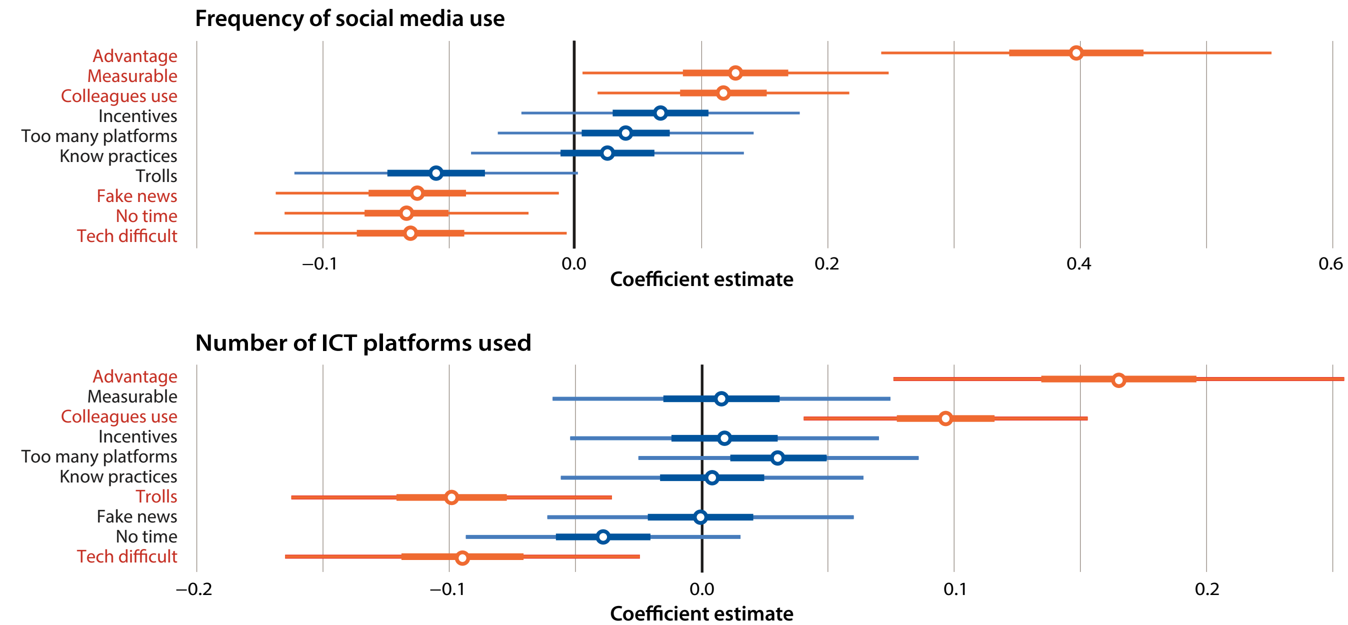

FIG. 3. Coefficient plots for innovation attribute regression model results. Each bar displays the coefficient estimate (bold dot) and the 50th (thick lines) and 95th (thin lines) percentile confidence intervals from the regression models. Any coefficient estimate below zero represents a negative correlation with the dependent variable, and above zero represents a positive correlation. The orange lines indicate coefficient estimates where the 95% confidence interval does not contain zero, which are statistically signficant at the $p < 0.05$ level according to standard null hypothesis tests.

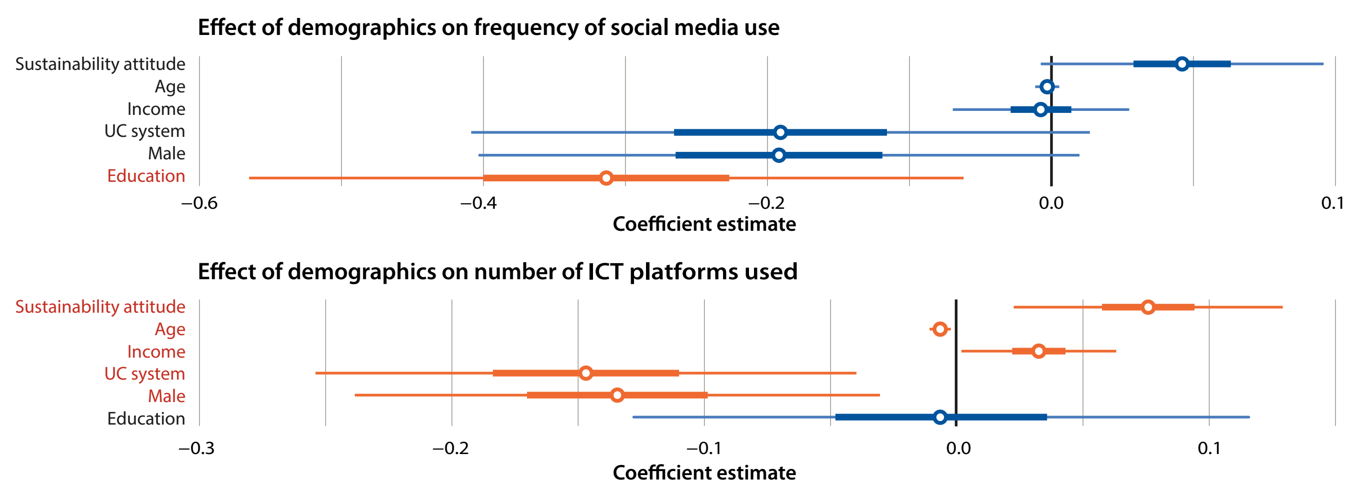

FIG. 4. Coefficient plots for demographic regression model results. Each bar displays the coefficient estimate (bold dot) and the 50th (thick lines) and 95th (thin lines) percentile confidence intervals from the regression models. Any coefficient estimate below zero represents a negative correlation with the dependent variable, and above zero represents a positive correlation. The orange lines indicate coefficient estimates where the 95% confidence interval does not contain zero, which are statistically signficant at the $p < 0.05$ level according to standard null hypothesis tests.

outside specialists with expertise in digital tools and measurement.

The risks of misinformation and credibility may be some of the most important for extension organizations to address at a strategic level. Such organizations typically desire to be perceived as impartial providers of evidence-based information. Social media platforms recognize that legitimate knowledge exists outside of Cooperative Extension but also provides a gateway for misinformation. At the individual level, the risk is not so much that a particular extension professional may make a mistake in communicating their own research, but rather they may unintentionally spread misinformation from others and be required to invest additional resources in sorting accurate social media information from misinformation. At the institutional level, social media's democratization of information creates the fear of messages being corrupted, misinterpreted or simply lost in the wash of real information and misinformation. In both cases, it is important to avoid damaging the reputation of providing high-quality science communication.

However, Bastos et al. (2018) provide some evidence that may mitigate these fears. Examining the topology of Twitter networks connected with UC Agriculture and Natural Resources Twitter users, Bastos et al. (2018) found that communities focused on specialized agricultural topics formed centralized networks in which a relatively small number of expert nodes collected and broadcast information to a large audience. In other words, relative to more general users and nonspecialists, technical experts become more central in the online networks and serve as important information hubs for specialized and technical issues. This suggests that social media communication is not completely incompatible with the traditional extension goal of providing hubs of expertise.

Further research is needed to increase confidence in our results and recommendations. More systematic comparison between extension professionals and their clientele would corroborate the extent and nature of the technology gap. While our findings are relevant for extension professionals involved in sustainable agriculture, it would be important to generalize the research to other types of agricultural sectors, compare different commodity groups, directly survey farmers and extend the research to natural resource managers. Using big data approaches to monitor the dynamics and effectiveness of communication is also an important effort going forward. CA

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RESEARCH ARTICLE

Broccoli meal fed to laying hens increases nutrients in eggs and deepens the yolk color

A study suggests 15% broccoli stem and leaves meal could be added to poultry diets without adverse effects on egg production or quality.

by Gabriela Pedroza, Thomas Famula and Annie King

Abstract

The nutritious stems and leaves of broccoli often go to landfills as byproducts after harvesting and processing of the florets. The stems and leaves contain specific carotenoids that are noted to have anti-allergic, anti-cancer and anti-obesity bioactivity. Research has shown the stems and leaves could be made into a meal, small amounts of which could be added to poultry diets to increase the nutrients in eggs and also deepen the color of the yolks. We studied adding a relatively high percentage (15%) of broccoli stem and leaves meal to the corn-soy diets of White Leghorn inbred crosses. Compared to the control group of hens, fed the unenhanced corn-soy diet, feed consumption, body weight, feed conversion, egg production, egg weight, albumen height, Haugh units and eggshell thickness were statistically similar. No harmful effects of the glucosinolates in broccoli were observed. Yolk color scores were significantly higher with the addition of the meal. The results justify larger studies with various commercial lines of laying hens and various levels of meal added to the diets.

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During the harvesting and processing of broccoli (*Brassica oleracea*), the florets are separated out for consumption, leaving the stems and leaves, an estimated 75% of the plant, to be returned to the soil (Richard Smith, UC Cooperative Extension, personal communication). In spite of their high levels of calcium, vitamins, coloring agents, carotenoids and antioxidants, the stems and leaves are often deposited in landfills (AgMRC 2017; USDA ARS 2015; Wu et al. 1992). U.S. governmental agencies have called for a reduction of this type of agricultural byproduct waste, to use it to feed more people and to reduce the quantities of it in landfills, which emit greenhouse gases that are harmful to the environment and ultimately to people (USDA and US EPA 2015). Instead of being wasted, broccoli stems and leaves could be turned into meal and added to poultry diets.

Broccoli contains carotenoids that are yellow, orange or deep red (these colors are invisible because they are masked by chlorophyll's green reflection of sunlight). Diets containing various carotenoids are fed to laying hens to deepen the yellow-orange color of the egg yolk, which is desired by consumers and food companies. Poultry farmers can use lutein in marigold and alfalfa extracts to produce darker yolks. Other carotenoids are found in corn and red pepper (Nimalaratne et al. 2012). Regulations permit use of synthetic carotenoids; however, they are expensive. Many researchers agree that discarded vegetables and fruits are good sources of natural carotenoids to increase yolk pigmentation (Calislar and Uygur 2010; Gonza et al. 1999; Hu et al. 2011; King and Griffin 2015; Lokaewmanee et al. 2010).

According to UC Davis researchers, adding broccoli stems and leaves meal to poultry diets can increase the amount of carotenoids in eggs.

Valerie Ozella

California produces 90% of the broccoli grown in the United States (AgMRC 2017; Le Strange et al. 2010). Feeding of some byproducts will need to follow regulatory guidelines (US FDA 2018). Limitations for use of various byproducts include legality of use, health hazards, anti-nutritional factors, consistency, ability to form pellets, palatability, quality and quantity of by-product and effect of feed on resultant quality of food (Chedly 2001).

Used as a carotenoid supplement in poultry diets, broccoli stems and leaves meal (BSLM), a no-cost by-product that has to be hauled to landfills, could be financially advantageous for both producers of conventionally produced or organic eggs. For consumers, there are also health benefits. Epidemiologic and animal studies revealed that carotenoids in diets are antioxidants, inhibit premalignant lesions and enhance immune responses. Specific dietary carotenoids also provide protection against macula degeneration (Kotake-Nara and Nagao 2011; Seddon 1994; Snodderly 1996). Some carotenoids, in particular, have attracted interest from health professionals due to their anti-allergic, anti-cancer and anti-obesity actions (Kotake-Nara and Nagao 2011). Specific carotenoids in broccoli have been shown to be less affected by heat (frying, boiling and heating by microwave), with losses ranging from 6% to 18% less than losses of other broccoli carotenoids (Bailey and Chen 1989; Karadas et al. 2006). In contrast to vegetable matrices, carotenoids in egg yolks are highly digestible and bioactive (Thierau et al. 2014; Zaripheh and Erdman 2002).

Used as a carotenoid supplement in poultry diets, broccoli stems and leaves meal (BSLM), a no-cost byproduct that has to be hauled to landfills, could be financially advantageous for both producers of conventionally produced or organic eggs.

Research suggests BSLM can only be a small part of poultry diets. Plants in the Brassica family possess secondary plant metabolites, glucosinolates, which have been shown to severely depress growth, reduce feed intake and decrease egg production if fed indiscriminately to poultry (Tripathi and Mishra 2007). However, Hu et al. (2011) fed laying hens diets with 0% to 9.0% BSLM and reported statistically similar egg production and egg quality measurements, and significantly darker yolk pigmentation with BSLM.

According to Hu et al. (2011), glucosinolates increase as the quantity of BSLM fed to layers increases. At 9%, there was 6.75×10^{-7}% of glucosinolate (based on molecular weight of methylglucosinolate) in the diet. This was well below the 1.62×10^{-6}% of glucosinolates in the diet considered detrimental to poultry health and production (Tripathi and Mishra 2007). If the amount of BSLM in the diet were 18%, twice that used by Hu et al. (2011), a linear association shows that the concentration of glucosinolates in the diet would be approximately 1.35×10^{-6}%, still below that reported to be harmful for poultry.

In a small study in the Department of Animal Science at UC Davis, we investigated the effects of increasing BLSM in poultry diets to 15%. Diets were fed to White Leghorn inbred crosses, similar in size and production characteristics to commercial layers. Our goal was to determine whether larger-scale research was warranted with various commercial layers and levels of BLSM in the diet.

Broccoli meal (BSLM) trial

All animal feeding and handling procedures in the study were approved by the Institutional Animal Care and Use Committee. Isoenergetic and isonitrogenous diets were formulated at UC Davis (Creative Formulation Concepts 2015, table 1). Diets were formulated as corn-soy (control) and the control plus 15% BSLM purchased at Harmony House (Franklin, N.C.). Diets were provided in three replicates to four hens in two runs of the experiment (table 1).

Hens were housed in individual cages (18 by 19 by 21 inches) in the same house, with a temperature range of 20°C to 25°C and 18 hours of light and 6 hours of darkness during each 24-hour cycle. Hens were allowed to acclimate to diets for approximately 4 days before measurements were taken; feed and water were provided *ad libitum* for 4 weeks.

Total carotenoids in diets were determined on pooled samples from each replication of the diet using

A formulated corn and soy feed, *right*, was the control in a trial to assess adding BSLM (broccoli stems and leaves meal), *left*, to a poultry diet. The BSLM increased egg yolk color with no detrimental effects on egg production or quality.

the method and equation described by de Carvalho and Gomes (2012). Weekly weights of hens were recorded. Eggs were collected daily, then labeled and stored at 4°C. Egg weight, albumen height, Haugh units, eggshell thickness and yolk color were determined within 3 days of egg collection. The Haugh unit, an overall measure of internal egg quality, was determined using the following equation:

$$HU = 100 * \log10\,(h - 1.7w0.37 + 7.6)$$

where h is height of albumen and w is egg weight (Eisen et al. 1962).

Eggshell thickness was determined following the method of Sun et al. (2012). Two measurements for thickness were made at the equatorial center of each eggshell using a digital caliper. A YolkFan (DSM Nutritional Products, Parsippany, N.J.) was used to rate yolk color relative to 15 standards. Ratings were obtained from separate blind measurements by two people.

All data, except eggshell thickness, were analyzed using R-3.2.1 (RStudio Team 2015). For the power analysis (12 birds per treatment, alpha of 0.05 and a power value of 0.8), the computed d value [(treatment 1 – treatment 2)/standard deviation] was 1.19, an estimate of the detectable difference in standard deviation units. Data were collected from a second run of the experiment with layers (a total of 24 for each treatment) to improve the accuracy of differences as calculated. Shell thickness was analyzed by ANOVA (SAS, Cary, N.C.). Means for all data were calculated and significant differences between treatments were determined for 95% accuracy ($P < 0.05$).

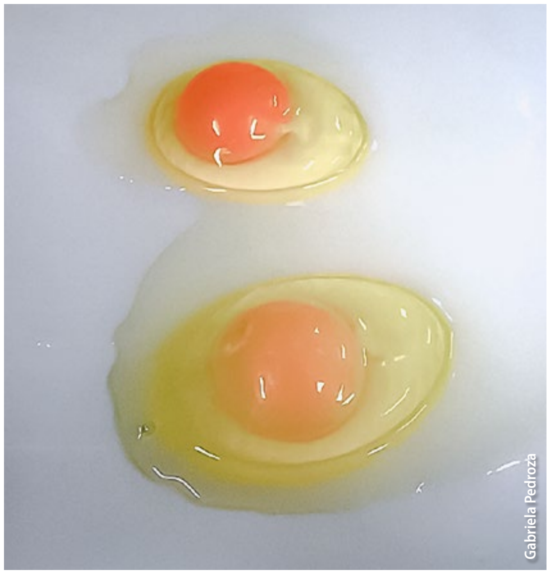
More carotenoids, deeper yolk color

Total carotenoids were statistically different at 1.11×10^{-3}, 2.56×10^{-4}, 4.10×10^{-5} and 9.77×10^{-5} pounds of total carotenoid per 100 pounds of BSLM, the control plus 15% BSLM, cornmeal, and a corn-soy commercial

TABLE 1. Composition of diets as fed

Ingredients	As fed	
	Control	Control + BSLM
	kg/20 kg, lb/44.4 lb	
Yellow dent corn grain	14.05	12.44
Soybean meal, no hull (48.5%)	4.22	2.93
Broccoli stems and leaves meal (dry)		3.00
Limestone, ground	1.33	1.31
Calcium phosphate dibasic	0.14	0.14
Vitamin mix	0.12	0.12
Mineral mix	0.05	0.05
Vegetable oil	0.07	0.014
Salt	0.014	0.002
Choline chloride		0.006
Ferrous sulfate	0.01	0.01
DL-methionine, 99%		0.001

Diets met the nutrient requirements of the NRC (1994).



Eggs laid by hens fed a diet enhanced with 15% BSLM (broccoli stems and leaves meal) had a darker yolk (top) than eggs laid by hens fed a diet without BSLM (bottom).

diet, respectively. The control plus 15% BSLM diet provided approximately 2.6 times more carotenoids than the commercial diet. BSLM has approximately 27 times more carotenoids when compared to cornmeal.

As shown in figure 1, color scores for egg yolks were significantly different throughout the trial. For the final 10 days, color scores were 7.70 ± 0.35 and 10.60 ± 0.36 for the control and control plus 15% BSLM, respectively.

After feeding BSLM in diets, Hu et al. (2011) reported yolk color scores of 9.25, 10.39, 10.92 and 11.28 for 0%, 3%, 6% and 9% BSLM, respectively. Our value for the control was lower than the value in that study for 0% BSLM. The difference may be due to the different groups of observers ranking differently or to hen strain, hen age or other factors. The similarity between the study's 9% BSLM score (Hu et al. 2011) and our score for 15% BSLM may indicate an upper limit for the deposition of carotenoids in egg yolk. Clearly, further investigation is warranted.

Egg production and quality maintained

Feed intake and weight gain were not significantly different for the two diets. The insignificant difference for the feed conversion ratio is shown in figure 2. Egg production, egg weight, albumen height and Haugh units were not affected by adding 15% BSLM to the commercial diet. Values for eggshell thickness were similar at 0.36 ± 0.05 and 0.37 ± 0.06 millimeters for the control and the experimental diet, respectively.

Nutritional impact, future research

Our study findings supported our hypothesis that 15% BSLM would not decrease hen growth, production and other egg quality measurements, but it would deepen the egg yolk color and greatly increase the carotenoids in eggs. Increasing the nutritional value of eggs would be especially important for people not consuming enough carotenoids from fruits and vegetables. Continued research on BSLM as a component of poultry diets promises to be important for U.S. consumption of carotenoids as well as for consumers in parts of the world where the nutrients in BSLM-enriched eggs would be important for overall improvements in health. Our results suggest that our research should continue with a full-scale study using commercial White Leghorns.

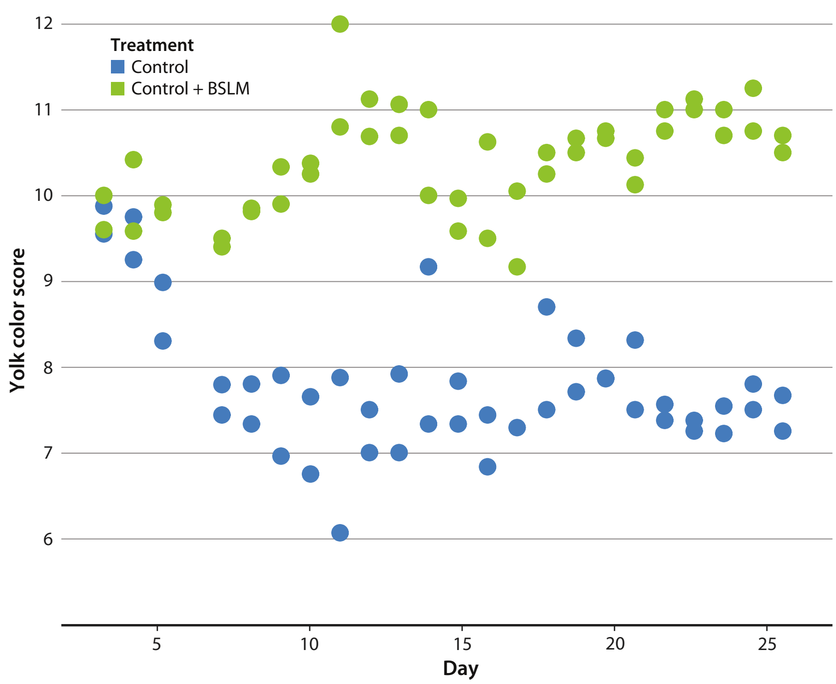


FIG. 1. A YolkFan was used to rate yolk color relative to 15 standards. The addition of BSLM (broccoli stems and leaves meal) to the poultry diet resulted in significantly higher scores.

As mentioned earlier, theoretically, almost 20% BSLM could be added to layer diets without detrimental effects on growth, production measurements and egg quality. Ongoing research will include (1) adding varying quantities (10% to 20%) of BSLM to diets of commercial layers and determining the corresponding concentration of specific carotenoids in the egg yolks, (2) examining the upper limit of carotenoid deposition and the medical effects of glucosinolates, and (3) determining the quantity of carotenoids in eggs before and after heating by various methods. CA

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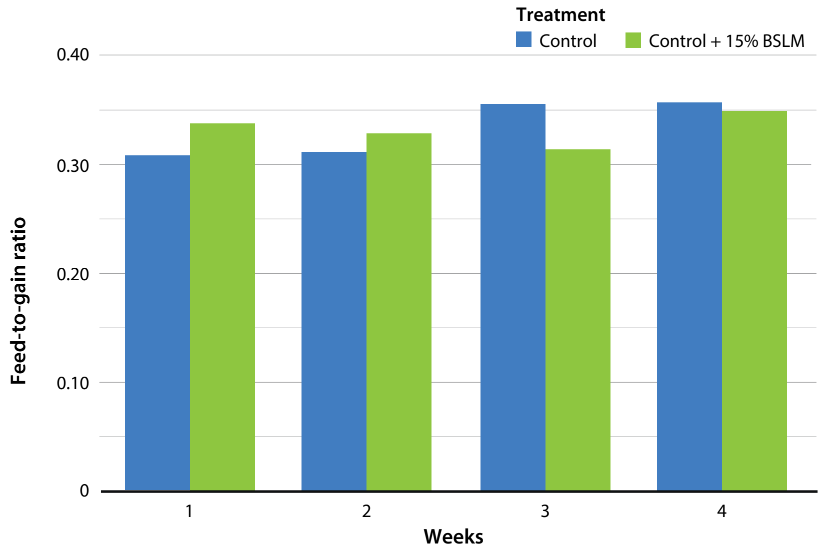

FIG. 2. Feed consumption and weight gained were determined weekly and the feed conversion ratio was calculated for the two treatments: the control, a commercial corn-soy feed, and the control plus 15% BSLM (broccoli stems and leaves meal). There was no significant difference between the treatments.

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Upcoming UC and UC ANR events

Sutter-Yuba and Colusa Almond Meetings

Sutter-Yuba: January 8, 2019, 9:00 a.m. to 11:00 a.m., Sutter County Ag Building, Yuba City
Colusa: January 17, 2019, 8:00 a.m. to 12:00 p.m., Granzella's Banquet Hall, Williams
Contact: Franz Niederholzer fjniederholzer@ucanr.edu or 530-218-2359

2019 San Joaquin Valley Grape Symposium

http://cefresno.ucanr.edu/?calitem=427568

Date: January 9, 2019
Time: 7:00 a.m. to 12:00 p.m.
Location: CPDES Hall, Easton
Contact: George Zhuang gzhuang@ucanr.edu or 559-241-7506

Merced Master Gardeners

Rose Pruning Workshop (Merced Master Gardeners)

https://ucanr.edu/?calitem=427683

Date: January 26, 2019
Time: 10:00 a.m. to 12:00 p.m.
Location: Applegate Park Rose Garden, Merced
Contact: UC Cooperative Extension Merced County 209-385-7403